**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C12N 15/11

A1

(11) International Publication Number: **WO 98/20160**

(43) International Publication Date: 14 May 1998 (14.05.98)

(21) International Application Number: PCT/US97/19896

(22) International Filing Date: 3 November 1997 (03.11.97)

(30) Priority Data:

| | | |
|---|---|---|
| 08/745,228 | 8 November 1996 (08.11.96) | US |
| 08/766,439 | 12 December 1996 (12.12.96) | US |

(71) Applicant *(for all designated States except US)*: E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HAZEL, James, William [US/US]; 1181 Liberty Grove Road, Conowingo, MD 21918 (US). JENSEN, Mark, Anton [US/US]; 1176 Fielding Drive, West Chester, PA 19382 (US).

(74) Agent: MAJARIAN, William, R.; E.I. Du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**

*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: GENETIC MARKERS AND METHODS FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA SPP*

(57) Abstract

A method, diagnostic sequences and primers are provided that are useful in identifying the *Listeria monocytogenes* and *Listeria spp*. The method involves identifying an RAPD–amplified DNA fragment common to *Listeria monocytogenes*, then identifying the most conserved regions of that DNA fragments, and the preparing specific primers useful for detecting the presence of a marker within the fragment whereby that set of primers is then useful in the identification of all *Listeria monocytogenes*. Markers within the same fragment that are specific to the *Listeria* genus are also identified and are useful for the identification of all *Listeria spp*.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AL** | Albania | **ES** | Spain | **LS** | Lesotho | **SI** | Slovenia |
| **AM** | Armenia | **FI** | Finland | **LT** | Lithuania | **SK** | Slovakia |
| **AT** | Austria | **FR** | France | **LU** | Luxembourg | **SN** | Senegal |
| **AU** | Australia | **GA** | Gabon | **LV** | Latvia | **SZ** | Swaziland |
| **AZ** | Azerbaijan | **GB** | United Kingdom | **MC** | Monaco | **TD** | Chad |
| **BA** | Bosnia and Herzegovina | **GE** | Georgia | **MD** | Republic of Moldova | **TG** | Togo |
| **BB** | Barbados | **GH** | Ghana | **MG** | Madagascar | **TJ** | Tajikistan |
| **BE** | Belgium | **GN** | Guinea | **MK** | The former Yugoslav Republic of Macedonia | **TM** | Turkmenistan |
| **BF** | Burkina Faso | **GR** | Greece | | | **TR** | Turkey |
| **BG** | Bulgaria | **HU** | Hungary | **ML** | Mali | **TT** | Trinidad and Tobago |
| **BJ** | Benin | **IE** | Ireland | **MN** | Mongolia | **UA** | Ukraine |
| **BR** | Brazil | **IL** | Israel | **MR** | Mauritania | **UG** | Uganda |
| **BY** | Belarus | **IS** | Iceland | **MW** | Malawi | **US** | United States of America |
| **CA** | Canada | **IT** | Italy | **MX** | Mexico | **UZ** | Uzbekistan |
| **CF** | Central African Republic | **JP** | Japan | **NE** | Niger | **VN** | Viet Nam |
| **CG** | Congo | **KE** | Kenya | **NL** | Netherlands | **YU** | Yugoslavia |
| **CH** | Switzerland | **KG** | Kyrgyzstan | **NO** | Norway | **ZW** | Zimbabwe |
| **CI** | Côte d'Ivoire | **KP** | Democratic People's Republic of Korea | **NZ** | New Zealand | | |
| **CM** | Cameroon | | | **PL** | Poland | | |
| **CN** | China | **KR** | Republic of Korea | **PT** | Portugal | | |
| **CU** | Cuba | **KZ** | Kazakstan | **RO** | Romania | | |
| **CZ** | Czech Republic | **LC** | Saint Lucia | **RU** | Russian Federation | | |
| **DE** | Germany | **LI** | Liechtenstein | **SD** | Sudan | | |
| **DK** | Denmark | **LK** | Sri Lanka | **SE** | Sweden | | |
| **EE** | Estonia | **LR** | Liberia | **SG** | Singapore | | |

TITLE

# GENETIC MARKERS AND METHODS FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA SPP*

This is a continuation-in-part of Application No. 08/745228, filed 8 November 1996.

## FIELD OF INVENTION

The invention relates to the field of molecular biology and the use of randomly amplified nucleic acid fragments (RAPD) for the selection of genetic markers useful in the identification of bacteria. More specifically, the invention relates to specific DNA marker sequences useful for the detection of *Listeria monocytogenes* and *Listeria spp.* and use of those diagnostic markers to determine if an unknown bacterium is a member of either *Listeria monocytogenes* or *Listeria spp.*

## BACKGROUND

Central to the field of microbiology is the ability to positively identify microorganisms at the level of genus, species or serotype. Correct identification is not only an essential tool in the laboratory, but it plays a significant role in the control of microbial contamination in the processing of food stuffs, the production of agricultural products, and the monitoring of environmental media such as ground water. Increasing stringency in regulations that apply to microbial contamination have resulted in a corresponding increase in industry resources which must be dedicated to contamination monitoring.

Of greatest concern is the detection and control of pathogenic microorganisms. Although a broad range of microorganisms have been classified as pathogenic, attention has primarily focused on a few bacterial groupings such as *Escherichia*, *Salmonella*, *Listeria* and *Clostridia*. Typically, pathogen identification has relied on methods for distinguishing phenotypic aspects such as growth or motility characteristics, and for immunological and serological characteristics. Selective growth procedures and immunological methods are the traditional methods of choice for bacterial identification and these can be effective for the presumptive detection of a large number of species within a particular genus. However, these methods are time consuming and are subject to error. Selective growth methods require culturing and subculturing in selective media, followed by subjective analysis by an experienced investigator. Immunological detection (e.g., ELISA) is more rapid and specific, however, it still requires growth of a significant population of organisms and isolation of the relevant antigens. For these reasons interest has turned to detection of bacterial pathogens on the basis of nucleic acid sequence.

It is well known, for example, that nucleic acid sequences associated with the ribosomes of bacteria are often highly conserved across genera and are therefore useful for identification (Webster, U.S. Patent No. 4,717,653 and U.S. Patent No. 5,087,558; Enns, *Lab. Med.*, 19, 295, (1988); Mordarski, *Soc. Appl. Bacteriol. Tech.* Ser., 20 (Chem. Methods Bact. Syst.), 41, (1985)). Weisburg et al. (EP 51736) disclose a method for the detection and identification of pathogenic microorganisms involving the PCR amplification and labeling of a target nucleotide for hybridization to 16S rDNA of *E. coli*. Lane et al. (WO 9015157) teach universal nucleic acid probes that hybridize to conserved regions of 23S or 16S rRNA of eubacteria.

Although bacterial ribosomal nucleic acids contain highly conserved sequences, they are not the only sources of base sequence conservation that is useful for microorganism identification. Wheatcroft et al. (CA 2055302) describe the selection of transposable elements, flanked by unique DNA sequences, for the detection of various *Rhizobium* strains. Similarly, Tommassen et al. (WO 9011370) disclose polynucleotide probes and methods for the identification and detection of gram-positive bacteria. The method of Tommassen et al. relies on probes corresponding to relatively short fragments of the outer membrane protein, OmpA, which is known to be highly conserved throughout gram-positive genera. Atlas et al. (EP 517154) teach a nucleic acid hybridization method for the detection of *Giardia sp.* based on designing probes with sequences complementary to regions of the gene encoding the giardin protein. Webster et al. (U.S. Patent No. 4,717,653) has expanded upon the use of rRNA in disclosing a method for the characterization of bacteria based on the comparison of the chromatographic pattern of restriction endonuclease-digested DNA from the unknown organism with equivalent chromatographic patterns of at least 2 known different organism species. The digested DNA has been hybridized or reassociated with ribosomal RNA information-containing nucleic acid from (or derived from) a known probe organism. The method of Webster et al. effectively establishes a unique bacterial nucleic acid "fingerprint" corresponding to a particular bacterial genus against which unknown "fingerprints" are compared.

Methods for the identification of *Listeria monocytogenes* on using specific hybridization probes or primers are known. For example, U.S. 5523205 and JP 05219997 teach DNA probes capable of hybridizing to a portion of the genome of pathogenic *Listeria* monocytogenes, but do not hybridize to genomes of other *Listeria* species. DE 4238699 and EP 576842 teach methods for detection of *Listeria monocytogenes* using primers designed to give amplification products specific to the *monocytogenes* genome. EP 576842 discusses a method for the

detection of *L. monocytogenes* using amplification primers based on genes encoding the highly conserved iap (invasion-associated protein) of *Listeria* and WO 9008841 teaches nucleic acid probes capable of hybridizing to ribosomal RNA (rRNA) or rDNA of *Listeria* and not to rRNA or DNA of non-*Listeria*.

The methods described above are useful for the detection of bacteria, but each relies upon knowledge of a gene, protein, or other specific sequence known *a priori* to be highly conserved throughout a specific bacterial group. An alternative method would involve a nontargeted analysis of bacterial genomic DNA for specific non-phenotypic genetic markers common to all species of that bacteria. For example, genetic markers based on single point mutations may be detected by differentiating DNA banding patterns from restriction enzyme analysis. As restriction enzymes cut DNA at specific sequences, a point mutation within this site results in the loss or gain of a recognition site, giving rise in that region to restriction fragments of different length. Mutations caused by the insertion, deletion or inversion of DNA stretches will also lead to a length variation of DNA restriction fragments. Genomic restriction fragments of different lengths between genotypes can be detected on Southern blots (Southern, *J. Mol. Biol.* 98, 503, (1975)). The genomic DNA is typically digested with any restriction enzyme of choice, the fragments are electrophoretically separated, and then hybridized against a suitably labeled probe for detection. The sequence variation detected by this method is known as restriction length polymorphism or RFLP (Botstein et al., *Am. J. Hum. Genet.* 342, 314, (1980)). RFLP genetic markers are particularly useful in detecting genetic variation in phenotypically silent mutations and serve as highly accurate diagnostic tools.

Another method of identifying genetic polymorphic markers employs DNA amplification using short primers of arbitrary sequence. These primers have been termed "random amplified polymorphic DNA" or "RAPD" primers (see Williams et al., *Nucl. Acids. Res.*, 18, 6531 (1990) and U.S. Patent No. 5,126,239; also EP 0 543 484 A2, WO 92/07095, WO 92/07948, WO 92/14844, and WO 92/03567). The RAPD method amplifies either double or single-stranded, nontargeted, arbitrary DNA sequences using standard amplification buffers, dATP, dCTP, dGTP and TTP, and a thermostable DNA polymerase such as *Taq*. The nucleotide sequence of the primers is typically about 9 to 13 bases in length, between 50 and 80% G + C in composition and contains no palindromic sequences. RAPD detection of genetic polymorphisms represents an advance over RFLP in that it is less time consuming, more informative, and readily susceptible to automation. Because of its sensitivity for the detection of polymorphisms, RAPD analysis and variations based on RAPD/PCR methods have become the methods of

choice for analyzing genetic variation within species or closely related genera, both in the animal and plant kingdoms. For example, Landry et al. (*Genome*, 36, 580, (1993)) discuss the use of RAPD analysis to distinguish various species of minute parasitic wasps that are not morphologically distinct. Van Belkum et al. (*Mol. Biochem Parasitol.*, 61, 69, (1993)) teach the use of PCR-RAPD for the distinction of various species of *Giardi*.

In commonly assigned U.S. Patent No. 5,340,728, Applicants disclosed a method of double-nested PCR which is used to detect the presence of a specific microbe. This disclosure first describes identifying a random, unique segment of DNA for each individual microorganism which will be diagnostic for that microorganism. To identify and obtain this diagnostic nucleic acid segment a series of polymorphic markers is generated from each organism of interest using single primer RAPD analysis. The RAPD series from each organism is compared to similarly generated RAPD series for other organisms, and a RAPD marker unique to all members of the group is then selected. The unique marker is then isolated, amplified and sequenced. Outer primers and inner primers suitable for double-nested PCR of each marker may then be developed. These primers comprise sequence segments within the RAPD markers, wherein the inner set of primers will be complementary to the 3' ends of the target piece of nucleic acid. These nested primers may then be used for nested PCR amplification to definitely detect the presence of a specific microorganism.

In commonly owned PCT U.S. 95/06704 (WO 95/33854), Applicants more particularly adapted and described this RAPD methodology to identify a sequence or marker. The presence of the marker is diagnostic for all individuals of the genus *Salmonella*. PCT U.S. 95/06704 teaches a method involving a RAPD amplification of genomic DNA of a representative number of *Salmonella* individuals to produce a RAPD amplification product, termed the diagnostic fragment. This diagnostic fragment must be present in the RAPD profiles in over 90% of the individuals tested. Sequence information from the diagnostic fragment enables identification of the most suitable PCR primer binding sites within the diagnostic fragment to define a unique diagnostic marker. Primers flanking this marker are useful for the generation of amplification products from *Salmonella* genomic DNA, but will not produce any amplification products in non-*Salmonella* genera.

In commonly owned USSN 08/608,881, Applicants disclose a method, diagnostic sequences and primers that are useful in the identification of the *Escherichia coli* 0157:H7 serotype. The method involves the identification of a RAPD-amplified DNA fragment common to 0157:H7 *Escherichia coli*, the

identification of the most conserved regions of that fragment, and the preparation of specific primers useful for detecting the presence of a marker within the fragment whereby that set of primers is then useful in the identification of all 0157:H7 *Escherichia coli*. The method of 08/608,881 does not teach markers useful for the specific identification of *Listeria monocytogenes* and *Listeria spp.*

A detection methodology using PCR/RAPD specific to *Listeria monocytogenes* and *Listeria spp.* would be of high utility in the food industry. Detection methods not dependent on sequences derived from a known gene or associated with a known phenotypic characteristic of *Listeria monocytogenes* and *Listeria spp.* have not previously been disclosed.

## SUMMARY OF THE INVENTION

The present invention provides a method for the specific identification of *Listeria monocytogenes* and *Listeria spp.* using diagnostic genetic markers.

A method is provided for determining whether an unknown bacterium is a *Listeria monocytogenes* that involves:

(A) amplifying genomic DNA from (i) a positive test panel of *Listeria monocytogenes* strains and (ii) a negative test panel of non- *monocytogenes Listeria* strains with a primer derived from a pre-marker diagnostic fragment for *Listeria monocytogenes* selected from the group of nucleic acids corresponding to SEQ ID NOS:17, 18, and 19 to yield a 1300 bp diagnostic fragment for each of the positive and negative test panels;

(B) selecting at least one *Listeria monocytogenes* diagnostic marker contained within the diagnostic fragment by comparing the diagnostic fragment obtained from the amplification of the positive test panel with the diagnostic fragment obtained from the amplification of the negative test panel whereby at least one highly conserved region in the diagnostic fragment of the positive test panel is identified which is less than 90% homologous to any member of the negative test panel;

(C) designing at least one amplification primer corresponding to the at least one diagnostic marker identified in step (B); and

(D) amplifying genomic DNA of the unknown bacterium under suitable annealing temperatures with the at least one amplification primer of step (C), whereby obtaining at least one amplification product indicates that the unknown bacterium is a *Listeria monocytogenes*.

The method preferably uses *Listeria monocytogenes* pre-marker diagnostic fragments selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:20-23. The method preferably uses *Listeria monocytogenes* diagnostic fragments that are at least 83% homologous to SEQ ID NOS:24-31 and

33-40 The method preferably uses diagnostic fragments selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:24-31 and 33-40 The method preferably uses at least one diagnostic marker selected in step (B) selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:46-83.

Preferably, the amplification primers used are about 15 to 30 bp in length and suitable annealing temperatures are in the range of about 60 °C - 70 °C.

A method is also provided for determining whether an unknown bacterium is a member of the genus *Listeria*, comprising

(A) amplifying genomic DNA from (i) a positive test panel of *Listeria monocytogenes* strains and (ii) a negative test panel of non-*monocytogenes* *Listeria* strains with a primer derived from a pre-marker diagnostic fragment for *Listeria monocytogenes* strains selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:17,18 and 19 to yield a 1300 bp diagnostic fragment for each of the positive and negative test panels;

(B) selecting at least one *Listeria* genus-specific diagnostic marker contained within the diagnostic fragment by comparing the diagnostic fragment obtained from the amplification of the positive test panel with the diagnostic fragment obtained from the amplification of the negative test panel whereby at least one highly conserved region in the diagnostic fragment of the positive test panel is identified which is at least 90% homologous to the corresponding positive test panel of diagnostic fragment;

(C) designing amplification primers corresponding to the at least one *Listeria* genus-specific diagnostic marker selected in step (B); and

(D) amplifying genomic DNA of the unknown bacterium under suitable annealing temperatures with the amplification primers of step (D), whereby obtaining amplification products indicates that the unknown bacterium is a member of the genus *Listeria*.

The genus-specific method at step (A) preferably uses a diagnostic fragment 83% homologous to any one of SEQ ID NOS:24-31 and 33-40. The method at step (A) preferably uses a diagnostic fragment selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:24-31 and 33-40. The method preferably uses *Listeria monocytogenes* pre-marker diagnostic fragments selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:20-23. The method preferably uses diagnostic markers selected in step (B) from the group consisting of nucleic acids corresponding to SEQ ID NOS:84-110. Preferably, the method uses amplification primers of about 15 to 30 bp in length and uses a suitable annealing temperature in the range of about 60 °C to 70°C.

A hybridization method for determining whether an unknown bacterium is a *Listeria monocytogenes* is provided comprising contacting the genomic DNA of the unknown bacterium with a nucleic acid probe selected from the group consisting of nucleic acid sequences corresponding to SEQ ID NOS:46-83, and then detecting hybridization of the nucleic acid probe with the genomic DNA. A genus-specific hybridization method for determining whether an unknown bacterium is a *Listeria monocytogenes* is provided comprising contacting the genomic DNA of the unknown bacterium with a nucleic acid probe selected from the group consisting of nucleic acid sequences corresponding to SEQ ID NOS:84-110, and then detecting hybridization of the nucleic acid probe with the genomic DNA.

Isolated nucleic acid fragments are provided selected from the group consisting of nucleic acid fragments corresponding to SEQ ID NOS:17 through 110. Isolated nucleic acid fragments are provided encoding the amino acid sequence as given in any one of SEQ ID NOS:32 and 41-45. This invention further provides isolated nucleic acid fragments having SEQ ID NOS:17-110

A further embodiment of the invention are nucleic acid fragments located on a diagnostic fragment of about 1300 bp and selected from the group consisting of nucleic acid fragments designated

1515(rc341x2)-26-363,
1515(rc341x2)-27-281,
1515-26-36,
1515-27-357,
1515-26-rc233,
1515(8585)-27-rc737,
1515(8585)-28-rc793
1515-30-76,
1515-30-88,
1515(8585)-30-624,
1515(8585)-30-rc483,
1515(8585)-30-rc555,
1515(8585)-30-rc573,
1515(8585)-30-rc824,

the diagnostic fragment characterized by

(A) at least 83% homology to any one of SEQ ID NOS:24-31 and 33-40; and

(B) an open reading frame of about 855 bp contained within the diagnostic fragment, the open reading frame encoding an amino acid sequence of any one of SEQ ID NOS:32 and 41-45

In greater detail, the methods involve the following steps:

(i) RAPD analysis: The genomic DNA of positive and negative test panels of a representative number of individuals for *Listeria monocytogenes* was amplified using RAPD primers. The positive test panel consisted of 20 strains of *Listeria monocytogenes* and the negative test panel consisted of 25 strains of non-*monocytogenes Listeria spp.* RAPD amplification gave some amplification products specific to the positive test panel that were not seen in the negative test panel.

The RAPD marker profiles from individuals of the positive test panel were compared with the RAPD marker profiles from individuals of the negative test panel and a nucleic acid fragment was selected where the fragment was present in all of the RAPD marker profiles from the positive test panel and absent in the RAPD marker profiles from the negative test panel. This fragment was termed a "pre-marker sequence".

(ii) Sequencing: The nucleotides of the pre-marker sequence of step (i) were sequenced to identify available primer binding sites.

(iii) Evaluation of the pre-marker sequence for *Listeria monocytogenes* specificity: Single primers derived from the pre-marker sequence were selected. These primers produced single amplification products when used to amplify genomic *Listeria monocytogenes* DNA.

(iv) Determination and isolation of the diagnostic fragment: Sequences of the flanking regions of the pre-marker sequence were determined revealing a diagnostic fragment of 1300 bp. Sequencing of the diagnostic fragment in *Listeria monocytogenes* and non-*monocytogenes Listeria* revealed conserved regions specific both to *Listeria spp.* in general and *Listeria monocytogenes* in particular. Amplification primers were designed based on these conserved regions.

(v) Preliminary selection of *Listeria monocytogenes* diagnostic primers on the basis of sensitivity to annealing temperature: Primers unique to *Listeria monocytogenes* were identified based on the diagnostic fragment. Primer pairs were selected on the basis of their ability to resist the formation of non-specific amplification products as annealing temperatures were reduced.

(vi) Final Selection of *Listeria monocytogenes* diagnostic primers: The primers of step (v) were used in the amplification of genomic DNA from a large group of *Listeria monocytogenes* (positive test panel) and non-

*monocytogenes* species (negative test panel) under specific annealing conditions, confirming the specificity of these primers for *Listeria monocytogenes* detection.

(vii) Preliminary Selectivity Testing for *Listeria spp.* diagnostic primers: Primers unique to *Listeria spp.* were identified based on the diagnostic fragment. Primer pairs were selected on the basis of their ability to specifically detect *L. spp.*

(viii) Selection of *Listeria spp.* diagnostic primers on the basis of sensitivity to annealing temperature: The primer pairs of step (vii) were screened on the basis of their ability to resist the formation of non-specific amplification products as the annealing temperatures were reduced.

(xi) Final Selection of *Listeria spp.* diagnostic primers: The primers of step (viii) were used in the amplification of genomic DNA from a large group of *Listeria spp.* (positive test panel) and non-*Listeria spp.* (negative test panel) under specific annealing conditions, confirming the specificity of these primers for *Listeria spp.* detection.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a gel showing RAPD patterns for *Listeria monocytogenes* strains comprising both the negative and positive test panels, amplified with the 12-mer primer 12CN015. The specific lanes are identified in Table 3.

Figure 2 shows a comparison of the amino acid sequences of *L. monocytogenes* #647, *L. innocua* DP #4450, *L. seeligeri* DP #3327, *L. welshimeri* DP #3359, and *L. ivanovii* DP #3340.

Figure 3 shows the unique *Listeria monocytogenes* specific diagnostic primer sequences located at 1515(rc341x2)-26-363, 1515(rc341x2)-27-281, 1515-26-36, 1515-27-357, 1515-26-rc233, 1515(8585)-27-rc737, and 1515(8585)-28-rc793 and a comparison of priming site sequences for strains representing the following species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. ivanovii*.

Figures 4A-4C are gels illustrating the appearance of anomalous and false positive amplification products as the annealing temperature was reduced for the 1515-26-36/1515-26-rc233 primer pair. The specific lanes are identified in Table 7.

Figure 5A displays the PCR product patterns of *Listeria monocytogenes* strains from the positive test panel amplified with the primer pair 1515-27-357/1515(8585)-27-rc737. The specific lanes are identified in Table 8, column A.

Figure 5B displays the PCR product patterns of *Listeria monocytogenes* strains from the negative test panel amplified with the primer pair

1515-27-357/1515(8585)-27-rc737 The specific lanes are identified in Table 8, column B

Figure 6 is a gel showing the seven *Listeria spp.* specific primer sequences located at 1515-30-76, 1515-30-88, 1515(8585)-30-624, 1515(8585)-30-rc483, 1515(8585)-30-rc555, 1515(8585)-30-rc573, and 1515(8585)-30-rc824 and a comparison of priming site sequences for strains representing the following species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. ivanovii*.

Figure 7 is a gel showing *Listeria spp.* positive test panel response for PCR products generated from primer set 1515-30-76/1515(8585)-30-rc555. The specific lanes are identified in Table 11.

Figure 8 is a gel showing *Listeria spp.* negative test panel response for PCR products generated from primer set 1515-30-76/1515(8585)-30-rc555. The specific lanes are identified in Table 12.

Applicants have provided 110 sequence listings in conformity with 37 C.F.R. 1.821-1.825 and Appendices A and B ("Requirements for Application Disclosure Containing Nucleotides and/or Amino Acid Sequences") and in conformity with "Rules for the Standard Representation of Nucleotide and Amino Acid Sequences in Patent Applications" and Annexes I and II to the Decision of the President of the EPO, published in Supplement No. 2 to the OJ EPO, 12/1992.

Sequences of SEQ ID NOS:1-16 are twelve-base arbitrary primers used in the generation of RAPD patterns. These are also shown in Table 1. Sequences of SEQ ID NOS:17-19 are single primers derived from the pre-marker sequences. These are also shown at Table 2. SEQ ID NOS:20 and 21 represent the pre-marker sequence for strain #647 and SEQ ID NOS:22 and 23 represent the pre-marker sequence for strain #1324. The amino acid composition for all *L. monocytogenes* strains is represented in SEQ ID NO:32. Sequences corresponding to SEQ ID NOS:24-31 and 33-40 are diagnostic fragments; Sequences corresponding to SEQ ID NOS:32 and 41-45 are open reading frames encoding amino acid sequences.

## DETAILED DESCRIPTION OF THE INVENTION

In the present method, Applicants have used RAPD amplification of *Listeria monocytogenes* and *Listeria spp.* genomic DNA to discover diagnostic fragments and primers useful for the specific detection of *Listeria monocytogenes* and *Listeria spp.* The fragments are used to generate specific primers from the most conserved regions for use in a PCR assay that will produce amplification products specific to either *Listeria monocytogenes* or *Listeria spp.*

Applicant's method is distinctive in the following regard. To selectively detect *Listeria monocytogenes* from all other *Listeria* or *Listeria spp.* from all other bacteria the method must be successful in determining the most conserved regions of the diagnostic fragments from a phenotypically uncharacterized segment of DNA common to all *Listeria monocytogenes* or all *Listeria spp.* One of skill in the art will recognize that conservation of sequence may be both an ally and an enemy in identifying the members of a particular genus. For example, many bacterial sequences are conserved across genera and these would not be useful in the determination of species within a particular genus. It is precisely for that reason that methods previously known in the art rely primarily on the analysis of sequences derived from proteins or genes known to be specific to a particular genus, i.e., ribosomal RNA or toxin-encoding genes. Applicant's method departs from the art in that the conserved sequences of the invention are not derived from a known gene nor is the sequence associated with any known phenotypic characteristic.

As used herein the following terms may be used for interpretation of the claims and specification.

"Nucleic acid" refers to a molecule which can be single-stranded or double-stranded, comprising monomers (nucleotides) containing a sugar, phosphate and either a purine or pyrimidine. In bacteria, lower eukaryotes, and in higher animals and plants, "deoxyribonucleic acid" (DNA) refers to the genetic material while "ribonucleic acid" (RNA) is involved in the translation of the information from DNA into proteins.

The term "primer-directed amplification" refers to any of a number of methods known in the art that result in logarithmic amplification of nucleic acid molecules using the recognition of a specific nucleic acid sequence or sequences to initiate an amplification process. Applicants contemplate that amplification may be accomplished by any of several schemes known in this art, including but not limited to the polymerase chain reaction (PCR) or ligase chain reaction (LCR). If PCR methodology is selected, the amplification method would include a replication composition consisting of, for example, nucleotide triphosphates, two primers with appropriate sequences, DNA or RNA polymerase and proteins. These reagents and details describing procedures for their use in amplifying nucleic acids are provided in U.S. Patent No. 4,683,202 (1987, Mullis et al.) and U.S. Patent No. 4,683,195 (1986, Mullis et al.).

The term "pre-marker sequence" refers to a 414 bp fragment of DNA that is an internal region of the diagnostic fragment.

The term "derived from", with reference to an amplification primer, refers to the fact that the sequence of the primer is a fragment of the sequence from which it has been "derived" The fragment is always denoted in a 5' to 3' orientation. The useful primer sequence size range for PCR amplification is about 15 base pairs to about 30 base pairs in length.

A "diagnostic fragment" refers to a particular DNA sequence which is highly conserved amongst the individuals of a particular genetically related population, for example, a genus, species, or serotype of bacteria. In the instant invention, the term "diagnostic fragment" is used to refer to the composite of that DNA fragment generated during RAPD amplification and those fragments that are generated from amplification with single primers derived from the pre-marker sequence, which are present in the RAPD and the single primer amplification profiles from either 1) all *Listeria monocytogenes* and absent from other *Listeria spp.* or 2) present in all *Listeria spp.* but absent in profiles from non-*Listeria* species. The term "diagnostic marker" is used herein to refer to that portion of the diagnostic fragment which can be targeted to produce an amplification product only in either *Listeria monocytogenes* or *Listeria spp.* The diagnostic marker is present only in the organism to be identified at the desired classification level (i.e., species or genus) and attempts to amplify the diagnostic markers in individuals not so targeted will give no amplification product. Within the context of the present invention diagnostic fragments which are diagnostic markers for *Listeria monocytogenes* and *Listeria spp.* and useful in Applicant's invention include nucleic acid sequences SEQ ID NOS:24-31 and 33-40 of about 1300 bp containing an open reading frame of 855 bp, encoding the peptide as given in SEQ ID NO:32.

The terms "conserved" or "highly conserved" refer to a level of similarity that exists between 2 or more nucleic acid fragments where there is at least 90% base similarity between the fragments. The term "base similarlity" refers to the relatedness between the nucelotide sequence of two nucleic acid molecules. Estimates of such similarity are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Sambrook et al., Molecular Cloning: A Laboratory Manual; volumes 1, 2, 3 (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York)).

The term "primer" refers to a nucleic acid fragment or sequence that is complementary to at least one section along a strand of the sample nucleic acid, wherein the purpose of the primer is to sponsor and direct nucleic acid replication of a portion of the sample nucleic acid along that strand. Primers can be designed to be complementary to specific segments of a targeted sequence. In PCR, for

example, each primer is used in combination with another primer forming a "primer set" or "primer pair"; this pair flanks the targeted sequence to be amplified. In RAPD amplification, single arbitrary primers are used to amplify nontargeted segments of nucleic acid which are located between the primer sequence sites in opposing DNA strands. The term "primer", as such, is used generally by Applicant to encompass any sequence-binding oligonucleotide which functions to initiate the nucleic acid replication process. "Diagnostic primers" will refer to primers designed with sequences complementary to primer binding sites on the diagnostic marker. Diagnostic primers are useful in the convenient detection and identification of diagnostic markers specific to *Listeria monocytogenes* and *Listeria spp.*

A "genetically related population" refers to any grouping of micro-organisms possessing multiple or single genotypic or phenotypic characteristics of sufficient similarity to allow said organisms to be classified as a single genus, species, or subspecies of bacteria. For purposes of the present disclosure, examples of genetically related populations include, for example, *Listeria monocytogenes* and *Listeria spp.*

A "test panel" refers to a particular group of organisms or individuals selected on the basis of their genetic similarity to each other or on the basis of their genetic dissimilarity to another group (i.e., another genus, species, subspecies or serotype). A "positive test panel" will refer to a number of individuals selected for the desired genetic similarity between those individuals and, in the instant case, will be comprised of individuals of either *Listeria monocytogenes* or *Listeria spp.*

Similarly, a "negative test panel" will refer to a test panel selected on the basis of genetic diversity between its members and the members of the positive test panel. A suitable negative test panel in the present invention would be comprised of non-*Listeria monocytogenes* where *L. monocytogenes* is the target organism or non-*Listeria spp.* where *Listeria spp.* is the target organism.

The term "unknown microorganism" or "unknown bacterium" is a microorganism or bacterium whose identity is undetermined.

The term "amplification product" refers to specific DNA fragments generated from any primer-directed nucleic acid amplification reaction. The diagnostic markers of the present invention are amplification products generated in PCR reaction using diagnostic primers and are useful for the detection of *Listeria monocytogenes* and *Listeria spp.*

The term "RAPD" refers to "random amplified polymorphic DNA". "RAPD amplification" refers to a method of single primer-directed amplification of nucleic acids using short primers of arbitrary sequence to amplify nontargeted,

random segments of nucleic acid. The method is disclosed and claimed in U S Patent No. 5,126,239 "RAPD method" or "RAPD analysis" refers to a method for the detection of genetic polymorphisms involving the nontargeted amplification of nucleic acids using short primers of arbitrary sequence, whereby the profile or pattern of "RAPD" amplification products is compared between samples to detect polymorphisms. "RAPD primers" refers to primers of about 8 to 13 bp, of arbitrary sequence, useful in the RAPD amplification or RAPD analysis according to the instant method. The "RAPD marker profile" refers to the pattern, or fingerprint, of amplified DNA fragments which are amplified during the RAPD method and separated and visualized by gel electrophoresis.

The term "ribotype" refers to a specific classification of bacteria or other microorganisms based on the digestion of genomic DNA with a restriction endonuclease, electrophoretic resolution of the restricted DNA and visualization of those fragments containing rDNA sequences by means of hybridization with a probe derived from the rDNA operon.

The diagnostic marker of the invention can be used to identify any member of either *Listeria monocytogenes* to the exclusion of other *Listeria spp.*, or *Listeria spp.* to the exclusion of all other bacteria. In the present invention, diagnostic primers flanking the marker are useful to amplify the marker using PCR. Alternatively, nucleic acid probes could be developed based upon some or all of the diagnostic marker sequences and thus used to detect the presence of the marker sequence using standard solid phase or solution nucleic acid hybridization and reporter methods. It is contemplated that regions of about 30 base pairs or more of the diagnostic marker, especially encompassing the primer regions could be used as sites for hybridization of diagnostic probes. These methods might be used specifically for the detection of *Listeria monocytogenes* or *Listeria spp.* in food, human or animal body fluids or tissues, environmental media or medical products and apparatti.

The instant method is more particularly described below with reference to the specific method steps as provided in the Summary of the Invention.

Selection of RAPD Primers and Detection of Diagnostic Fragment in Members of the Positive and Negative Test Panels, step (i):

Genomic DNA isolated from positive and negative test panels of microorganisms was subjected to RAPD amplification using sixteen 12-base primers of arbitrary sequence. The positive test panel consisted of 20 strains of *Listeria monocytogenes* and is described in detail in the GENERAL METHODS section below. The negative test panel consisted of a variety of 25 *Listeria spp.* and is also described in the GENERAL METHODS section below. Techniques

for the isolation of genomic DNA are common and well known in the art and examples may be found in Sambrook et al., Molecular Cloning: A Laboratory Manual - volumes 1, 2, 3 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)).

RAPD primers of 12 bases in length were used because at this primer length the RAPD patterns generally contained one to five amplified DNA fragments. Use of shorter primers frequently resulted in a large number of amplification products, which made the extraction of a single homogeneous fragment for sequencing much more difficult. When primers of greater than 12 bases were used a significant fraction of the bacterial strains produced no RAPD products which would have necessitated the screening of a much larger number of arbitrary primers. One of the primers, designated 12CN015 (Table I, GENERAL METHODS), was found to produce a 414 bp amplification product (termed a pre-marker sequence) in all of the positive test panel. 12CN015 had the sequence of GGA CAG AGC ATA (SEQ ID NO:15). The primer, 12CN15, was found to produce a 414 bp amplification product in all *L. monocytogenes* strains. This 414 bp pre-marker sequence was not observed in the amplification products of the negative test panel using the same primer. Examples of the 12CN15 RAPD patterns for strains of both test panels is shown in Figure 1.

Sequencing of Pre-marker Sequence, step (ii):

Since the 414 bp product was unique to *L. monocytogenes*, samples of this product were isolated for two strains different of *L. monocytogenes*, DP #647 and DP #1324, and the respective products sequenced. The two strains represented a ribotype that was highly polymorphic (#1324) based on RAPD patterns and a ribotype that is pathogenic (#647). The object of selecting a common pathogenic strain and a polymorphic strain of *L. monocytogenes*, was to characterize the genetic diversity likely to be found within the 12CN15 marker fragment.

The complete sequences of the 414 bp products for DP #647 and #1324, including the flanking 12CN15 sequences, are shown in SEQ ID NOS:20 and 21 for DP #647 and SEQ ID NO:21 and 23 for DP #1324. Comparison of the DP #647 and #1324 sequences shows a 98% homology. Both sequences appear to be an internal section of an open reading frame (ORF) with the same amino acid composition.

Evaluation of Pre-marker Sequence for *Listeria monocytogenes* Specificity, step (iii):

The purpose of the initial PCR screening was to identify sequence domains that demonstrated species selectivity. Primers based on the 414 bp pre-marker sequences were first evaluated for their ability to specifically amplify from *L. monocytogenes* genomic DNA. Initial primer sequences were 26 bases long

with a GC composition of 50 ± 5% to allow for an annealing temperature in the range of 70 °C. Priming sites were selected within a distance of 200 bases from each 12CN15 priming site. These sequences were examined to insure that inter- and intra-primer interactions were minimized to avoid the production of nonspecific PCR artifacts.

Many of the initial primer pairs that were tested generated multiple amplification products from genomic *L. monocytogenes* DNAs. Multiple PCR products can occur when at least 8 bases of one 3' primer sequence appears as an inverted repeat in the region adjacent to the originally targeted priming sites. To determine which products were the result of inverted repeats of a single primer sequence and which primers were responsible for these products, amplification reactions were run using single primers. A significant number of single primers such as 1515-26-85, (SEQ ID NO:17) 1515-26-rc292 (SEQ ID NO:18) and 1515-26-rc341 (SEQ ID NO:19) (Table 2) were capable of generating PCR products in single primer amplifications.

Determination and Isolation of the Diagnostic Fragment, step (iv):

Determination of sequences adjacent to the RAPD marker

Each of the single primers, 1515-26-85, (SEQ ID NO:17) 1515-26-rc292 (SEQ ID NO:18) and 1515-26-rc341 (SEQ ID NO:19) generated a PCR product that contained part of the original 414 bp fragment plus additional sequence. Sequence determination of these augmented CN15 fragments was accomplished by means well known in the art.

Sequencing revealed approximately 900 bases of additional sequence. The new sequence consisted of 400 bases in the region preceding the original 12CN15 site and 500 bases in the region following the second 12CN15 site revealing a complete open reading frame (ORF).

The degree of conservation within this genetic locus was further examined by amplification of additional *Listeria monocytogenes* strains and comparison of those amplification products with the strains already sequenced.

Comparison revealed that all strains contain a 1300 bp diagnostic fragment containing a complete open reading frame of 855 bases. This sequence was used as the diagnostic fragment and is identified by SEQ ID NOS:24-31. To determine if this fragment contained selective priming sites for *Listeria monocytogenes* specific primers, further analysis of the sequence composition was done to determine if it was distinct from other *Listeria* spp.

BASE SIMILARITY COMPARISON BETWEEN 1300BP DIAGNOSTIC FRAGMENTS

A comparison of the base similarity between all isolated 1300 bp diagnostic fragments is given below.

| | #647 | #1324 | #4450 | #654 | #3327 | #3340 |
|---|---|---|---|---|---|---|
| L. MONO #647 | 97.1% | 86.8% | 84.5% | 83.3% | 83.0% | |
| L. MONO #1324 | | 86.6% | 84.4% | 83.6% | 82.7% | |
| L. INN. #4450 | | | 85.9% | 84.3% | 82.9% | |
| L. WELSH. #654 | | | 83.2% | 82.9% | | |
| L. SEEL. #3327 | | | | 86.1% | | |
| L. IVAN. #3340 | | | | | | |

Sequencing marker fragment for other *Listeria* species - determination of sequence composition of diagnostic fragment

Evaluation of primer sites in the 1300 bp diagnostic fragment showed that not all primer sites were specific for *Listeria monocytogenes*, suggesting conservation of the sequence composition at the genus level.

The genetic composition was explored by isolating and sequencing the 855 bp fragment and the flanking regions from a panel of non-*monocytogenes* strains. All strains were found to contain the 855 base open-reading frame. However, a comparison of the sequences demonstrated there were species-dependent differences in the nucleic acid composition within the ORF SEQ ID NOS:33-40 and Table 3, Example 3). The specific locations of these differences suggest priming sites where the diversity of nucleotide sequence may be sufficient to allow for selective priming of *Listeria monocytogenes* strains.

Preliminary selection of *Listeria monocytogenes* diagnostic primers on the basis of sensitivity to annealing temperature, step (v):

A comparison of sequence data for the five *L. spp.* made it possible to identify seven priming sites that were unique to *L. monocytogenes*. These priming sites were as follows; 1515(rc341x2)-26-363, 1515(rc341x2)-27-281, 1515-26-36, 1515-27-357, 1515-26-rc233, 1515(8585)-27-rc737, and 1515(8585)-28-rc793 (Figure 3) 26 to 30 bases in length depending on the GC composition. Longer primers were used for sites with a GC composition of < 50% to assure that a 70 °C annealing temperature could be maintained. Figure 7 shows the primer sequences and a comparison of priming site sequences for strains representing the following species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. ivanovii*.

To minimize the likelihood that *L. monocytogenes* selective primers would generate PCR products from mismatched priming in non-*monocytogenes* strains,

the relationship between annealing temperature and selectivity was evaluated. A test panel consisting of both *Listeria monocytogenes* and non-*monocytogenes* strains was prepared and amplifications were carried out over a range of temperatures and analyzed for the appearance of amplification products from *Listeria spp.* that were non-*monocytogenes*. Primer pairs that showed an onset of non-specific amplification at or above 65 °C were considered as unsuitable for use as *Listeria monocytogenes* diagnostic primers.

Final selection of *Listeria monocytogenes* diagnostic primers, step (vi):

Before making the final primer selection, the accuracy of all candidate primer sets was evaluated for a larger group of *L. monocytogenes* strains. A set of 323 strains of *L. monocytogenes* was used to evaluate the inclusivity of the following primer sets: 1) 1515-27-357/1515(8585)-27-rc737; 2) 1515-27-357/1515(8585)-28-rc793; and 3) 1515(rc341x2)-26-(-363)/1515-26-rc233 (Figure 3). Accuracy in detection of *Listeria monocytogenes* ranged from 99.5% to 100% depending on the primers and amplification conditions used. None of the three primer sets generated amplification products for the 30 non-*monocytogenes* strains in the annealing stringency test panel.

Preliminary selectivity testing of *Listeria spp.* diagnostic primers, step (vii):

A comparison of sequence data for the five above-mentioned *Listeria* species made it possible to identify seven priming sites present in strains representing these species that were at least 90% homologous to *L. monocytogenes*. These priming sites were as follows: 1515-30-76, 1515-30-88, 1515(8585)-30-624, 1515(8585)-30-rc483, 1515(8585)-30-rc555, 1515(8585)-30-rc573, and 1515(8585)-30-rc824 (Figure 6). Primers to these sites were made 30 bases in length to compensate for mismatches in sequence and to assure that a 70 °C annealing temperature could be maintained.

Before evaluating stringency conditions, the accuracy of all candidate primer sets was evaluated for a group of 33 *L. spp.* strains. These strains were used to evaluate the inclusivity of the following primer sets:

1) 1515-30-76/1515(8585)-30-rc483; 2) 1515-30-76/1515(8585)-30-rc555;
3) 1515-30-76/1515(8585)-30-rc573; 4) 1515-30-76/1515(8585)-30-rc824;
5) 1515-30-88/1515(8585)-30-rc483; 6) 1515-30-88/1515(8585)-30-rc555;
7) 1515-30-88/1515(8585)-30-rc573;8) 1515(8585)-30-624/1515(8585)-30-rc824.

The accuracy in the detection of *L. spp.* was 100% for the following primer sets: 1515-30-76/1515(8585)-30-rc555;1515-30-76/1515(8585)-30-rc573; 1515-30-88/1515(8585)-30-rc555; and 1515-30-88/1515(8585)-30-rc573.

Selection of *Listeria spp.* diagnostic primers on the basis of sensitivity to annealing temperature, step (viii):

As with *Listeria monocytogenes*, the relationship between annealing temperature and selectivity was evaluated to minimize the generation of non-specific amplification products. This test was performed for all the primer pairs that scored 100% in the preliminary inclusivity evaluation. Amplifications were carried out on a test panel of 7 strains of *Listeria spp.* and non-*L. spp.* at an annealing temperature of 70 °C. Annealing temperature was then decreased in 5 °C increments until the onset of nonspecific amplification products. The 1515-30-76/1515(8585)-30-rc555 primer set was found to be the least likely to generate nonspecific amplification products.

Final selection of *Listeria spp.* diagnostic primers, step (ix):

Before confirming the selection of the 1515-30-76/1515(8585)-30-rc555 primer set, the accuracy of this primer set was evaluated for a group of 73 *L. spp.* strains. Accuracy in detection of *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. ivanovii* was 100%. This primer set did not generate amplification products for the 65 non-*Listeria spp.* strains in the non-*Listeria* test panel.

To determine whether the selected primer set would generate PCR products from mismatched priming in non-*monocytogenes* strains, the relationship between annealing temperature and selectivity was evaluated. A test panel consisting of both *Listeria spp.* and non-*L. spp.* strains was prepared and amplifications were carried out over a range of temperatures and analyzed for the appearance of amplification products from non-*L. spp.* In this fashion, it was determined that 60 °C is the lowest annealing temperature that will result in specificity for *L. spp.*, and 70 °C is the preferred temperature that should be used when using this set of *Listeria spp.* specific primers.

## EXAMPLES

### GENERAL METHODS

Procedures for DNA amplifications and other protocols common in the art of molecular biology used in the following examples may be found in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in the following examples may be found in Manual of Methods for General Bacteriology (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American Society for Microbiology, Washington, DC. (1994) or in the work of Thomas D. Brock (in

Biotechnology: A Textbook of Industrial Microbiology, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA). All reagents and materials used for the growth and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "mL" means milliliters, "L" means liters.

DNA sequencing was performed by Lark Sequencing Technologies Inc., (Houston, Texas) or according to the method of Sanger et al. (*Proc. Natl. Acad. Sci., USA* 74, 5463, (1977)) using fluorescence-labeled dideoxynucleotides and the Genesis 2000™ DNA Analysis System (E. I. du Pont de Nemours and Company, Wilmington, DE).

Construction of Positive and Negative Test Panels

*Listeria monocytogenes*

A positive test panel consisting of 20 genotypically different *Listeria monocytogenes* strains was constructed for the identification of a *Listeria monocytogenes* and *Listeria spp.* RAPD marker. The *Listeria monocytogenes* strains of the postive test panel encompassed all commonly encountered serotypes and included *L. monocytogenes*, DP #647, #899, #1324 and #3386.

The negative test panel consisted of 25 different *Listeria* non-*monocytogenes* strains comprising the species *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. grayi*, *L. murrayi* and *L. ivanovii*.

RAPD Primers

RAPD primers used for amplification of genomic DNA from the positive and negative test panels are given below in Table 1.

TABLE 1

Twelve-Base Arbitrary Primers Used in the Generation of RAPD Patterns

| | | | |
|---|---|---|---|
| 12CN01- | AGC TGA TGC TAC SEQ ID NO:1 | 12CN09- | AAC CTC GTG TAG SEQ ID NO:9 |
| 12CN02- | AGT CGA ACT GTC SEQ ID NO:2 | 12CN10- | CAT TCG GGT ACA SEQ ID NO:10 |
| 12CN03- | TTA GTC ACG GCA SEQ ID NO:3 | 12CN11- | GCC CTT AGT GAA SEQ ID NO:11 |
| 12CN04- | TGC GAT ACC GTA SEQ ID NO:4 | 12CN12- | GCA GTT ATG ACC SEQ ID NO:12 |
| 12CN05- | CTA CAG CTG ATG SEQ ID NO:5 | 12CN13- | CCA GCT ATC TCT SEQ ID NO:13 |
| 12CN06- | GTC AGT CGA ACT SEQ ID NO:6 | 12CN14- | AGA AGG CAG TTG SEQ ID NO:14 |
| 12CN07- | GGC ATT AGT CAC SEQ ID NO:7 | 12CN15- | GGA CAG AGC ATA SEQ ID NO:15 |
| 12CN08- | CGT ATG CGA TAC SEQ ID NO:8 | 12CN16- | CGT TTC GCT TCA SEQ ID NO:16 |

Primer Nomenclature:

Primers names identified in the following examples are derived in the following manner: The first number, 1515, indicates the primer sequence comes from the RAPD fragment primed at both ends by 12CN15. The second number indicates the primer length. The third number identifies the 3' base position of the primer, where position 1 is at the 5' end of the original putative 12CN15 priming site. The rc designation means that the priming site is located on the complementary strand.

Primer locations may also be referred to by the last number of the identifying number. Thus "1515-26-85" may be referred to as "85".

RAPD primers may be referred to with out the "12" designation. Thus "12CN15" may also be referred to as "CN15".

The primers described below in Table 2 are derived from the pre-marker sequence. As single primers they match the pre-marker sequence and they also match at least the last 9 bases of 3' sequence at another location outside of the pre-marker sequence. The net effect is that these primers will generate amplification products in single primer reactions from genomic DNA. These products contain part of the original 414 bp fragment plus additional sequence.

TABLE 2

| *SPECIFICITY* | *I.D.* | *SEQUENCE* | *SEQ ID NO* |
|---|---|---|---|
| *Pre-marker* | 1515-26-85 | TGC TGT TTG GTT TGC TCT AGC CCA GTG | SEQ ID NO: 17 |
| | 1515-26-rc292 | CAA CTT TCC ACA TGG CGC GAT TAT TTG | SEQ ID NO: 18 |
| | 1515-26-rc341 | GGG GAA CTG CCG AAG ATC GTA CAG CA | SEQ ID NO: 19 |

## EXAMPLE 1

## ISOLATION OF PRE-MARKER SEQUENCE FROM *LISTERIA MONOCYTOGENES* BY RAPD ANALYSIS

Example 1 details the isolation of the 414 bp pre-marker sequence from *Listeria monocytogenes* genomic DNA using Random Amplified Polymorphic DNA RAPD primer analysis.

A set of eight 12-base primers (Table I) was used in a RAPD analysis of 20 strains of *Listeria monocytogenes*. The results of these amplifications were examined for a *Listeria monocytogenes*-specific amplification product that could be easily separated from other RAPD products.

The amplification and data acquisition protocol for each 12-base primer RAPD reaction was as follows:

Amplification protocol: For each 50 μL reaction, 1.5 μL - dNTP mix (5 mM dNTP each), 36.3 μL - deionized water, 5 μL - 10X reaction buffer (500 mM KCl, 100 mM tris @ pH 8.3, 15 mM $MgCl_2$, 0.003% gelatin), 5 μL - primer (10 mM), 0.4 μL - Taq polymerase (5 U/μL), and 1.2 μL - Taq dilution buffer (10 mM tris @ pH 8.0 and 1.0% Tween 20) were combined. A 1.0 μL aliquot of genomic bacterial DNA @ 50 ng/μL was added to each mixture. The reaction was heated to 94 °C for 2 min. Twenty-eight cycles of the following temperature profile were run: 15" @ 94 °C, 5' @ 46 °C, 2' ramp to 72 °C, and 1' @ 72 °C. At the end of cycling the reaction was incubated at 72 °C for 7 min.

After amplification a 5 μL aliquot of the reaction was combined with 2 μL of Ficol-loading buffer and run on a 4% acrylamide gel (29:1)/1.0x TBE. Follow-ing electrophoresis, the gels were stained with ethidium bromide. The stained gels were placed on a transilluminator and electronically imaged with a high sensitivity CCD camera. Images were stored in computer memory for subsequent analysis.

Analysis: The primer, 12CN15, (SEQ ID NO:15) was found to produce a 414 bp (SEQ ID NO:32) amplification product in all *L. monocytogenes* strains. This 414 bp fragment was not observed in the amplification products of the negative test panel using the same primer. Examples of the 12CN15 RAPD patterns for strains of both test panels is shown in Figure 1. The lanes are correlated to Figure 1 as follows in Table 3:

TABLE 3

| Lane | Strain | Lane | Strain |
|---|---|---|---|
| 1 | *L. welshimeri #943* | 13 | *L. ivanovii #1165* |
| 2 | *L. monocytogenes #945* | 14 | *L. monocytogenes #1281* |
| 3 | *L. seeligeri #949* | 15 | *L. monocytogenes #1287* |
| 4 | *L. innocua #950* | 16 | *L. seeligeri #1290* |
| 5 | *L. monocytogenes #1047* | 17 | *L. monocytogenes #1283* |
| 6 | *L. seeligeri #1059* | 18 | *L. monocytogenes #1295* |
| 7 | *L. seeligeri #1061* | 19 | *L. monocytogenes #1299* |
| 8 | *L. monocytogenes #1068* | 20 | *L. seeligeri #1303* |
| 9 | *L. monocytogenes #1069* | 21 | *L. monocytogenes #1313* |
| 10 | *L. monocytogenes #1070* | 22 | *L. seeligeri #1317* |
| 11 | *L. innocua #1089* | 23 | *L. monocytogenes #1324* |
| 12 | *L. innocua #1157* | 24 | *L. monocytogenes #1963* |

* Arrows denote 414 bp product in *L. monocytogenes* strains. (See Lanes 2, 5, 8, 10, 14, 15, 18, 19, 21 and 24 of Figure 1.)

As is evident from Figure 1, the positive test panel produced a characteristic amplification product of 414 bp which appeared in all of the 20 *Listeria monocytogenes* strains tested. Additionally it is seen that none of the negative test panel group showed the 414 bp amplification product seen in the positive test panel.

## EXAMPLE 2

## GENERATION OF THE DIAGNOSTIC FRAGMENT FROM SEQUENCE FLANKING THE PRE-MARKER SEQUENCE

Example 2 illustrates the sequencing of the flanking regions of the 414 bp pre-marker sequence using single primers and the generation of the diagnostic fragment.

The 414 bp pre-marker sequence of Example 1 was commercially sequenced from two strains of *Listeria monocytogenes* (#647 and #1324) described above. The pre-marker sequence for each strain is given in SEQ ID NOS:20 and 21 for #647 and SEQ ID NOS:22 and 23 for #1324.

Primers were designed based on the pre-marker sequence, and evaluated for their ability to specifically amplify from *Listeria monocytogenes* genomic DNA. Initial primer sequences were 26 bases long with a GC composition of 50 ± 5% to allow for an annealing temperature in the range of 70 °C. Priming sites were selected within a distance of 200 bases from each 12CN15 priming site. Following this method three single primers were identified as 1515-26-85 (SEQ ID NO:17), 1515-26-rc292 85 (SEQ ID NO:18), and 1515-26-rc341 85 (SEQ ID NO:19), (Table 2) which generated a PCR product in the absence of a second primer.

Each of these single primers generated a PCR product that contained part of the original pre-marker sequence plus additional sequence. Sequence of these augmented 12CN15 fragments was accomplished using the chain-termination method of Sanger et al. (*Proc. Natl. Acad. Sci., USA* 74, 5463, (1977)) using fluorescence-labeled dideoxynucleotides and the Genesis 2000™ DNA Analysis System (E. I. du Pont de Nemours and Company, Wilmington, DE).

The new sequence consisted of 400 bases in the region preceding the original 12CN15 site and 500 bases in the region following the second 12CN15 site. This sequence data made it possible to identify a complete ORF plus several hundred bases upstream and downstream from the reading frame.

To further characterize the degree of conservation within this genetic locus, nucleic acid sequences were determined for two additional strains of *L. monocytogenes*, DP #899 and DP #3386. DP #899 is an additional representative of a known pathogenic *L. monocytogenes* ribotype group.

DP #3386 is a strain of a less pathogenic *L. monocytogenes* ribotype group Sequencing of these strains was accomplished using the chain-termination method of Sanger et al., (supra) and the Genesis 2000™ DNA Analysis System. The complete sequences for the *L. monocytogenes* strains, DP #647, #899, #1324 and #3386, are shown in SEQ ID NOS.24-31. (The 5' end of the original CN15 priming site was designated as base number 1 to provide a common reference point for all of the sequence data.)

Analysis of the nucleic acid sequence showed that all four strains contained a complete open reading frame of 855 bases. Within this ORF DP #647, #899, and #3386 all showed identical nucleic acid sequences. DP #1324 is 97% homologous with the other three *L. monocytogenes* strains. When the nucleic acid sequences were translated into the corresponding amino acid sequence DP #1324 was found to be identical to the other three strains. The amino acid composition for all of the *L. monocytogenes* strains is shown in SEQ ID NO:32).

The promoter and the terminal spacer sequences for DP #647, #899, and #3386 were also identical. DP #1324 shows an homology of 98% and 96% with the promoter and the terminal spacer, respectively. This genetic locus and the surrounding region showed a high level of conservation among strains of *L. monocytogenes*.

Further analysis of the sequence composition of the 1300 bp diagnostic fragment containing the 855 bp ORF was needed to determine if it was sufficiently distinct from other *L. spp* so as to provide selective priming sites for a PCR-based assay for *L. monocytogenes*.

## EXAMPLE 3

## DETERMINATION OF SEQUENCE COMPOSTITION OF DIAGNOSTIC FRAGMENT BY COMPARISON OF MARKER FRAGMENT SEQUENCE WITH OTHER *LISTERIA* SPECIES

A preliminary evaluation of primer sites in the augmented CN15 marker demonstrated that many locations did not discriminate between *L. monocytogenes* and other *L. spp*. This observation suggested that much of the sequence composition of this genetic locus was conserved at the genus level. To determine whether this genetic locus contained any sequences that were unique to *L. monocytogenes*, strains representing *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. ivanovii* were also selected for sequencing. (Marker sequences were not determined for *L. grayi* and *L. murrayi* because these species were considerably more polymorphic than other *L. spp*.) Previously determined non-selective priming sites were used to generate quantities of DNA suitable for sequencing. As with *L. monocytogenes*, primers 1515-26-85, (SEQ ID NO:17), 1515-26-rc292

(SEQ ID NO:18), and 1515-26-rc341 (SEQ ID NO:19), used in single primer amplifications also generated PCR products suitable for sequencing. Sequencing was accomplished using the chain-termination method of Sanger et al., and the Genesis 2000™ DNA Analysis System.

A comparison of the nucleotide sequences showed that each of the above species contained an 855 base open-reading frame. However, there were some species dependent differences in the nucleic acid composition within the ORF. Sequences SEQ ID NOS:33-40 show the nucleic acid composition for the following strains: *L. innocua* DP #4450, *L. seeligeri* DP #3327, *L. welshimeri* DP #3359, and *L. ivanovii* DP #3340, respectively. These differences in nucleotide sequence are responsible for corresponding species variations in amino acid sequence. The nucleotide and amino acid sequence homology is summarized in Table 4. Figure 2 shows a comparison of the amino acid sequences of each *L. spp.*, including *L. monocytogenes*.

TABLE 4
Open Reading Frame Nucleotide and Amino Acid Sequence Comparison between *Listeria* Species

| Species | Nucleotide Homology to *L. monocytogenes* #647 | Amino Acid Homology to *L. monocytogenes* #647 |
|---|---|---|
| *L. innocua* | 86.7% | 95.8% |
| *L. seeligeri* | 84.5% | 94.0% |
| *L. welshimeri* | 83.8% | 93.7% |
| *L. ivanovii* | 83.3% | 93.3% |

Of the *L. spp.* that were tested, *L. innocua* clearly showed the greatest similarity to *L. monocytogenes*. The remaining three species all showed roughly comparable levels of divergence in nucleotide and amino acid composition.

The promoter and terminal spacer nucleic acid sequences also showed significant differences between species. The homologies of promoter and spacer sequences compared to *L. monocytogenes* are summarized in Table 5.

TABLE 5
Promoter and Terminal Spacer Sequence Comparison between *L. Spp.*

| Species | Promoter Homology to *L. monocytogenes* #647 | Terminal Spacer Homology to *L. monocytogenes* #647 |
|---|---|---|
| *L. innocua* | 91.9% | 79.4% |
| *L. seeligeri* | 85.0% | 75.4% |
| *L. welshimeri* | 90.9% | 75.5% |
| *L. ivanovii* | 83.7% | 84.0% |

The homology to *L. monocytogenes* found in promoter and terminal spacer sequences is significantly different from that observed for the open reading frame sequences. *L. innocua* and *L. welshimeri* both show homologies to *L. monocytogenes* in the promoter region that are significantly greater than the corresponding ORF homology. For *L. seeligeri* and *L. ivanovii* the promoter regions and ORF regions both show comparable levels of homology with *L. monocytogenes*. In three species the terminal spacer sequences show a notable decrease in homology compared to the promoter regions and open reading frames, while in *L. ivanovii* similar levels of homology to *L. monocytogenes* are found in all three regions.

## EXAMPLE 4

## SELECTION OF *LISTERIA MONOCYTOGENES* DIAGNOSTIC PRIMERS ON THE BASIS OF SENSITIVITY TO ANNEALING TEMPERATURE

Identification of Priming sites and Primer synthesis:

A comparison of sequence data for the five *L. spp.* (Example 3) made it possible to identify seven priming sites that were unique to *L. monocytogenes*. These priming sites were as follows: 1515(rc341x2)-26-363, 1515(rc341x2)-27-281, 1515-26-36, 1515-27-357, 1515-26-rc233, 1515(8585)-27-rc737, and 1515(8585)-28-rc793 (Figure 3). "Uniqueness" is defined as any sequence region of 26-30 bases where the homology between *L. monocytogenes* and any other *Listeria spp.* is less than 90%.

Primers were synthesized to correspond to these sites and ranged in length from 26 to 30 bases depending on the GC composition. Longer primers were used for sites with a GC composition of <50% to assure that a 70 °C annealing temperature could be maintained.

Figure 3 shows the primer sequences and a comparison of priming site sequences for strains representing the following species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. ivanovii*.

Determination of annealing termperature:

To minimize the likelihood that *L. monocytogenes*-selective primers would generate PCR products from mismatched priming in non-*monocytogenes* strains, the relationship between annealing temperature and selectivity was evaluated. A 33-strain test panel consisting of 15 *L. innocua*, 5 *L. welshimeri*, 6 *L. seeligeri*, 3 *L. ivanovii*, 1 *L. grayi* and 3 *L. monocytogenes* was prepared. Amplifications were first carried out at an annealing temperature of 70 °C. Annealing temperature was then decreased in 5 °C increments until amplification products began to appear for the non-*monocytogenes* strains. The amplification and detection protocol is summarized below.

Combine 1.5 μL - dNTP mix (5 mM each dNTP), 40 μL - deionized water, 5 μL - 10X reaction buffer (500 mM KCl, 100 mM tris @ pH 8.3, 15 mM $MgCl_2$, 0.003% gelatin) 0.4 μL - Taq polymerase (5 U/μL), 1.2 μL - Taq dilution buffer (10 mM tris @ pH 8.0 and 1.0% Tween 20), 0.66 μL - each primer (10 μM), and 1.0 μL - genomic DNA @ 50 ng/μL. Heat to 94 °C for 2 min. Thirty-five cycles of the following temperature profile were run: 15"@ 94 °C; 2' at the specified annealing temperature and 1' @ 72 °C. At the conclusion of cycling the reaction was incubated at 72 °C for 7 min.

Visualization of amplification products is the same as described above.

Table 6 shows a comparison of the onset of false positive responses as the annealing temperature is reduced.

TABLE 6

Percent False Positive *L. monocytogenes* Responses as a Function of Annealing Temperature

| | Annealing Temperature, C. | | | |
|---|---|---|---|---|
| Primer Sets | 70° | 65° | 60° | 55° |
| 1515-27-357/ 1515(85,85)-27-rc737 | 0% | 0% | 5% | ND |
| 1515-27-357/ 1515(85,85)-28-rc793 | 0% | 0% | 55% | ND |
| 1515-26-36/ 1515-26-rc233 | 0% | 5% | 27% | ND |
| 1515(rc341,rc341)-27-(-281)/ 1515-26-rc233 | 0% | 0% | 0% | 1.5% |
| 1515(rc341,rc341)-26-(-363)/ 1515-26-rc233 | 0% | 0% | 0% | 33% |

* Primer label numbers contained in parentheses, i.e., (85, 85) and (rc341, rc341), indicate that these primers were derived from fragments generated by the single primers, 1515-26-85 and 1515-26-rc341.

Primer pairs that produced no false positives at 5 °C below the standard annealing temperature of 70 °C were considered as candidates for a PCR-based *L. monocytogenes* assay. Of all the primer sets tested, only 1515-26-36/ 1515-26-rc233 showed an unacceptable onset of false positive responses at 65 °C.

Figures 4A-4C are gels illustrating the appearance of anomalous amplification products as the annealing temperature was reduced for the 1515-26-36/ 1515-26-rc233 primer pair. The gel lanes are identified as follows in Table 7:

TABLE 7

| Lane | Strain | Lane | Strain |
|---|---|---|---|
| 1 | *L. seeligeri #2874* | 17 | *L. innocua #3429* |
| B | BLANK | 18 | *L. ivanovii #3357* |
| 2 | *L. innocua #2921* | 19 | *L. welshimeri #3558* |
| 3 | *L. ivanovii #3072* | 20 | *L. innocua #3571* |
| 4 | *L. innocua #3241* | 21 | *L. innocua #3797* |
| 5 | *L. innocua #3244* | 22 | *L. seeligeri #3828* |
| 6 | *L. seeligeri #3327* | 23 | *L. innocua #4094* |
| 7 | *L. ivanovii #3340* | 24 | *L. innocua #4101* |
| 8 | *L. innocua #3352* | 25 | *L. innocua #4323* |
| 9 | *L. welshimeri #3354* | B | BLANK |
| 10 | *L. grayi #3356* | 26 | *L. seeligeri #4333* |
| 11 | *L. welshimeri #3359* | 27 | *L. innocua #4442* |
| 12 | *L. seeligeri #3371* | 28 | *L. innocua #4450* |
| 13 | *L. seeligeri #3374* | 29 | *L. innocua #4452* |
| B | BLANK | 30 | *L. innocua #4463* |
| 14 | *L. welshimeri #3411* | 31 | *L. monocytogenes #3847* |
| 15 | *L. welshimeri #3412* | 32 | *L. monocytogenes #4324* |
| 16 | *L. innocua #3420* | 33 | *L. monocytogenes #4341* |

## EXAMPLE 5

## FINAL EVALUATION OF *LISTERIA MONOCYTOGENES* DIAGNOSTIC PRIMERS

Example 5 illustrates the inclusiveness of the identified *Listeria monocytogenes* diagnostic primers for all strains of *Listeria monocytogenes*.

The accuracy of all candidate primer sets was evaluated for a larger group of *L. monocytogenes* strains. A set of 323 strains of *L. monocytogenes* was used to evaluate the inclusivity of a set of three primer sets. PCR assay conditions were the same as those specified above except that only a 70 °C annealing temperature was used. In PCR-based assays using these three primer sets, the accuracy was as follows:

1) 1515-27-357/1515(8585)-27-rc737, 100%;
2) 1515-27-357/1515(8585)-28-rc793, 99.2%; and
3) 1515(rc341x2)-26-363/1515-26-rc233, 99.5%.

Although primer -281/rc233 was not tested, its inclusivity response was expected to be comparable to 1515(rc341x2)-26-363/1515-26-rc233. None of the three primer sets generated amplification products for the 30 non-*monocytogenes*

strains in the annealing stringency test panel. Examples of the positive and negative test panel results for the 1515-27-357/1515(8585)-27-rc737 primer pair are shown in Figures 5A and 5B respectively. Figures 5A and 5B each show a gel analysis which corresponds to the strains listed in Table 8

TABLE 8

| A | Positive test panel response for PCR products generated from primer set *1515-27-357/1515(8585)-27-rc737* | B | Negative test panel response for PCR products generated from primer set *1515-27-357/1515(8585)-27-rc737* |
|---|---|---|---|
| Lane | Strain | Lane | Strain |
| 1 | *L. monocytogenes #652* | 1 | *L. seeligeri #2874* |
| 2 | *L. monocytogenes #1049* | B | BLANK |
| 3 | *L. monocytogenes #936* | 2 | *L. innocua #2921* |
| 4 | *L. monocytogenes #954* | 3 | *L. ivanovii #3072* |
| 5 | *L. monocytogenes #957* | 4 | *L. innocua #3241* |
| 6 | *L. monocytogenes #952* | 5 | *L. innocua #3244* |
| 7 | *L. monocytogenes #946* | 6 | *L. seeligeri #3327* |
| 8 | *L. monocytogenes #955* | 7 | *L. ivanovii #3340* |
| 9 | *L. monocytogenes #937* | 8 | *L. innocua #3352* |
| B | BLANK | 9 | *L. welshimeri #3354* |
| 10 | *L. monocytogenes #1051* | 10 | *L. grayi #3356* |
| 11 | *L. monocytogenes #1046* | 11 | *L. welshimeri #3359* |
| 12 | *L. monocytogenes #1067* | 12 | *L. seeligeri #3371* |
| 13 | *L. monocytogenes #1055* | 13 | *L. seeligeri #3374* |
| 14 | *L. monocytogenes #1057* | B | BLANK |
| 15 | *L. monocytogenes #1087* | 14 | *L. welshimeri #3411* |
| 16 | *L. monocytogenes #1145* | 15 | *L. welshimeri #3412* |
| 17 | *L. monocytogenes #1146* | 16 | *L. innocua #3420* |
| 18 | *L. monocytogenes #1153* | 17 | *L. innocua #3429* |
| B | BLANK | 18 | *L. ivanovii #3357* |
| 19 | *L. monocytogenes #1144* | 19 | *L. welshimeri #3558* |
| 20 | *L. monocytogenes #1322* | 20 | *L. innocua #3571* |
| 21 | *L. monocytogenes #1287* | 21 | *L. innocua #3797* |
| 22 | *L. monocytogenes #1316* | 22 | *L. seeligeri #3828* |
| 23 | *L. monocytogenes #1306* | 23 | *L. innocua #4094* |
| 24 | *L. monocytogenes #1298* | 24 | *L. innocua #4101* |
| 25 | *L. monocytogenes #1302* | 25 | *L. innocua #4323* |
| 26 | *L. monocytogenes #1285* | B | BLANK |
| 27 | *L. monocytogenes #1286* | 26 | *L. seeligeri #4333* |

| | | | |
|---|---|---|---|
| 8 | BLANK | 27 | *L. innocua #4442* |
| 28 | *L. monocytogenes #1294* | 28 | *L. innocua #4450* |
| 29 | *L. monocytogenes #1283* | 29 | *L. innocua #4452* |
| 30 | *L. monocytogenes #1288* | 30 | *L. innocua #4463* |
| 31 | *L. monocytogenes #1284* | 31 | *L. monocytogenes #3847* |
| 32 | *L. monocytogenes #1282* | 32 | *L. monocytogenes #4324* |
| 33 | *L. monocytogenes #1464* | 33 | *L. monocytogenes #4341* |

## EXAMPLE 6

## SELECTIVITY TESTING FOR *LISTERIA SPP.* DIAGNOSTIC PRIMERS

### Selection of *Listeria spp.* Diagnostic Primers

A comparison of sequence data for the five *Listeria* species made it possible to identify priming sites that were at least 90% homologous to *L. monocytogenes*. The following priming sites were selected as possible candidates: 76, 88, 624, rc483, rc555, rc573, and rc324. Primers to these sites were made 30 bases in length to compensate for mismatches in sequence and to assure that a 70 °C annealing temperature could be maintained. Figure 6 shows the primer sequences and a comparison of priming site sequences for strains representing the following species: *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri* and *L. ivanovii*. Primer pairs from this group were first evaluated with a 33-strain test panel consisting of 15 *L. innocua*, 5 *L. welshimeri*, 6 *L. seeligeri*, 3 *L. ivanovii*, 1 *L. grayi* and 3 *L. monocytogenes*. Amplifications were first carried out at an annealing temperature of 70 °C. The results of this evaluation are summarized in Table 9.

TABLE 9
% Positive Response for PCR-Based Assay of *L. spp.*:
Effect of Priming Site Location

| Priming Sites: | 76 | 88 | 624 |
|---|---|---|---|
| rc483 | 80% | 85% | Not Applicable |
| rc555 | 100% | 100% | Not Applicable |
| rc573 | 100% | 100% | Not Applicable |
| rc824 | 0% | Not Determined | 0% |

The 76, 88, rc555 and rc573 priming sites all appear to be viable at a 70 °C annealing temperature, based on the 100% positive response that is achieved with any combination of these primers. When rc483 is used in conjunction with 76 or 88 the positive response drops off to the 80-85% response range. Decreasing the annealing temperature to 65 °C increases the response to 97%. Although both 76/rc824 and 624/rc824 generated no PCR product with a 70 °C annealing

temperature, when the temperature was decreased to 65 °C the positive response increased to 97%. In all of the tests at 65 °C the only false negative was a single L. *grayi* strain. It appears that the melting temperatures of the rc824 and rc483 primers are too low to permit their effective use at a 70 °C. No further testing was done with these primers. The 76, 88, rc555 and rc573 priming sites all appear to be viable at a 70 °C annealing temperature.

*L. grayi* and *L. murrayi* are not as frequently encountered as other *L. spp.* However, since no sequence data was determined for these species, additional strains were tested with the primer sets that scored 100%. The test group consisted of 7 strains of *L. grayi* and 4 strains of *L. murrayi*. The positive response of the various primer sets was as follows:

76/rc555 50%;
76/rc573 18%;
88/rc555 27%; and
88/rc573 9%.

The weak and variable response of *L. grayi* and *L. murrayi* to the genus level priming sites is not surprising. DNA-DNA hybridization studies have shown that *L. grayi* and *L. murrayi* were only moderately related to reference strains of *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. ivanovii*, i.e., at 3-29% and 1-9% respectively. (Rocourt et al., *Curr. Microbiol.* 7:383-388 (1982).) Based on this and other DNA-DNA hybridization studies, it has been suggested that *L. grayi* and *L. murrayi* are sufficiently different from *L. monocytogenes* to merit their separation into a new genus, *Murraya grayi*. The issue of how these species should be classified is currently undecided. Regardless of how strains from these two species are ultimately classified, the genus level primers show a generally weak positive response to strains from this group. The strength of the positive response is expected to be extremely dependent on the level of DNA used in the assay with sensitivity to *L. grayi* and *L. murrayi* strains expected to run 3 orders of magnitude poorer than other *Listeria* species.

## EXAMPLE 7

## SELECTION OF *LISTERIA SPP.* DIAGNOSTIC PRIMERS ON THE BASIS OF SENSITIVITY TO ANNEALING TEMPERATURE

To minimize the likelihood that *L. spp.* primers would generate PCR products from mismatched priming in non-*Listeria* strains, the relationship between annealing temperature and amplification specificity was evaluated. Amplifications were carried out on a test panel of 7 strains at an annealing temperature of 70 °C. Annealing temperature was then decreased in 5 °C increments until the onset of nonspecific amplification products. The primer

combinations of 76/rc573 and 88/rc573 both showed nonspecific products at 65 °C. At 60 °C 88/rc555 began to show nonspecific products. Such products were not observed for the 76/rc555 primer set until the annealing temperature was reduced to 55 °C. Since the 76/rc555 primer set was least likely to generate nonspecific amplification products this primer set was the primary candidate for the *Listeria spp.* detection assay.

## EXAMPLE 8

## FINAL EVALUATION OF *LISTERIA SPP.* DIAGNOSTIC PRIMERS

### Final Evaluation of *Listeria spp.* Candidate Primer Set

Before the 76/rc555 primer selection was confirmed, the accuracy of this set was evaluated for additional *L. spp.* strains. This entire test panel consisted of 73 strains that were broken down by species as follows: 9 *L. monocytogenes*, 34 *L. innocua*, 5 *L. welshimeri*, 11 *L. seeligeri*, 3 *L. ivanovii*, 7 *L. grayi* and 4 *L. murrayi*. PCR conditions were the same as those specified above except that only a 70 °C annealing temperature was used. All of the strains belonging to the species *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. ivanovii*, tested at 100% positive for the 76/rc555 primer set. As reported above, the test group of 7 *L. grayi* and 4 *L. murrayi* strains scored at 50%.

The accuracy of the 76/rc555 primer set was also evaluated on a non-*Listeria spp.* test panel consisting of 65 strains that represent a variety of related gram positive strains and enteric gram negative strains. The species tested are summarized in Table 10.

TABLE 10
Negative Test Panel for Screening of *Listeria spp.* Primer Set
1515-30-76/1515(8585)-30-rc555

| Species | No. of Strains | Species | No. of Strains |
|---|---|---|---|
| *Aeromonas species* | 1 | *Salmonella enteritidis* | 3 |
| *Bacillus cereus* | 4 | *Salmonella redlands* | 1 |
| *Bacillus subtilis* | 2 | *Salmonella virchow* | 1 |
| *Bacillus thuringiensis* | 3 | *Salmonella santiago* | 2 |
| *Carnobacterium piscicola* | 2 | *Staphylococcus aureus* | 4 |
| *Enterococcus casseliflavus* | 1 | *Staphylococcus carnosus* | 2 |
| *Enterococcus faecalis* | 10 | *Staphylococcus epidermidis* | 6 |
| *Enterococcus faecium* | 1 | *Staphylococcus species* | 1 |
| *Enterococcus species* | 1 | *Staphylococcus warneri* | 2 |
| *Escherichia coli* | 2 | *Staphylococcus xylosus* | 1 |
| *Lactococcus lactis* | 9 | *Brochothrix thermosphacta* | 8 |

None of the strains in Table 10 produced a positive PCR response for annealing temperatures ranging from 60-70 °C. Examples of the results of *Listeria spp.* and non-*Listeria spp.* test panels for the 76/rc555 primer pair are shown by gel analysis in Figures 7A and 7B respectively. The lanes are identified as follows in Table 11:

TABLE 11

*Listeria spp.* positive test panel response for PCR products generated from primer set 1515-30-76/1515(8585)-30-rc555 corresponding to Figure 4

| Lane | Strain | Lane | Strain |
|---|---|---|---|
| 1 | *L. seeligeri #2874* | 17 | *L. innocua #3429* |
| B | BLANK | 18 | *L. ivanovii #3357* |
| 2 | *L. innocua #2921* | 19 | *L. welshimeri #3558* |
| 3 | *L. ivanovii #3072* | 20 | *L. innocua #3571* |
| 4 | *L. innocua #3241* | 21 | *L. innocua #3797* |
| 5 | *L. innocua #3244* | 22 | *L. seeligeri #3828* |
| 6 | *L. seeligeri #3327* | 23 | *L. innocua #4094* |
| 7 | *L. ivanovii #3340* | 24 | *L. innocua #4101* |
| 8 | *L. innocua #3352* | 25 | *L. innocua #4323* |
| 9 | *L. welshimeri #3354* | B | BLANK |
| 10 | *L. grayi #3356* | 26 | *L. seeligeri #4333* |
| 11 | *L. welshimeri #3359* | 27 | *L. innocua #4442* |
| 12 | *L. seeligeri #3371* | 28 | *L. innocua #4450* |
| 13 | *L. seeligeri #3374* | 29 | *L. innocua #4452* |
| B | BLANK | 30 | *L. innocua #4463* |
| 14 | *L. welshimeri #3411* | 31 | *L. monocytogenes #3847* |
| 15 | *L. welshimeri #3412* | 32 | *L. monocytogenes #4324* |
| 16 | *L. innocua #3420* | 33 | *L. monocytogenes #4341* |

TABLE 12

*Listeria spp.* negative test panel response for PCR products generated from primer set 1515-30-76/1515(8585)-30-rc555

| Lane | Strain | Lane | Strain |
|---|---|---|---|
| 1 | *Escherichia coli #642* | 23 | *Lactococcus lactis #3587* |
| 2 | *Staphylococcus epidermidis #764* | 24 | *Lactococcus lactis #3588* |
| 3 | *Staphylococcus epidermidis #783* | 25 | *Lactococcus lactis #3589* |
| 4 | *Staphylococcus aureus #789* | 26 | *Lactococcus lactis #3590* |
| 5 | *Staphylococcus epidermidis #796* | 27 | *Lactococcus lactis #3591* |
| 6 | *Staphylococcus warneri #797* | 28 | *Escherichia coli #3803* |
| 7 | *Staphylococcus warneri #799* | 29 | *Lactococcus lactis #3817* |
| 8 | *Staphylococcus aureus #895* | 30 | *Aeromonas species #3818* |
| 9 | *Carnobacterium piscicola #920* | | BLANK |
| 10 | *Staphylococcus aureus #923* | 31 | *Enterococcus faecalis #3837* |
| | BLANK | 32 | *Enterococcus faecalis #3838* |
| 11 | *Bacillus subtilis #1011* | 33 | *Enterococcus species #4095* |
| 12 | *Bacillus subtilis #1041* | 34 | *Enterococcus faecium #4428* |
| 13 | *Staphylococcus carnosus #1090* | 35 | *Salmonella redlands #4563* |
| 14 | *Staphylococcus carnosus #1091* | 36 | *Salmonella enteritidis #4565* |
| 15 | *Staphylococcus xylosus #1120* | 37 | *Salmonella enteritidis #4593* |
| 16 | *Carnobacterium piscicola #1160* | 38 | *Bacillus thuringiensis #4941* |
| 17 | *Bacillus thuringiensis #1221* | 39 | *Bacillus thuringiensis #5083* |
| 18 | *Staphylococcus aureus #2095* | 40 | *Enterococcus faecalis #5504* |
| 19 | *Enterococcus faecalis #3074* | 41 | *Salmonella virchow #5508* |
| 20 | *Lactococcus lactis #3584* | 42 | *Enterococcus casseliflavus #5574* |
| | BLANK | | BLANK |
| 21 | *Lactococcus lactis #3585* | 43 | *Listeria monocytogenes #3844* |
| 22 | *Lactococcus lactis #3586* | 44 | *Listeria monocytogenes #3278* |

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HAZEL, JAMES WILLIAM
JENSEN, MARK ANTON

(ii) TITLE OF INVENTION: GENETIC MARKERS AND METHODS FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA SPP.*

(iii) NUMBER OF SEQUENCES: 110

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: E. I. DU PONT DE NEMOURS AND COMPANY
(B) STREET: 1007 MARKET STREET
(C) CITY: WILMINGTON
(D) STATE: DELAWARE
(E) COUNTRY: U.S.A.
(F) ZIP: 19898

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.50 INCH DISKETTE
(B) COMPUTER: IBM PC COMPATIBLE
(C) OPERATING SYSTEM: MICROSOFT WINDOWS 3.1
(D) SOFTWARE: MICROSOFT WORD 2.0C

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPALICATION DATA:
(A) APPLICATION NUMBER: 08/745,228
(B) FILING DATE: NOVEMBER 8, 1996

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FLOYD, LINDA AXAMETHY
(B) REGISTRATION NO.: 33,692
(C) REFERENCE/DOCKET NUMBER: MD-1065-A

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 302-892-8112
(B) TELEFAX: 302-773-0164

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCTGATGCT AC 12

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGTCGAACTG TC 12

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAGTCACGG CA 12

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGCGATACCG TA 12

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTACAGCTGA TG 12

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTCAGTCGAA CT 12

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCATTAGTC AC 12

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGTATGCGAT AC 12

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AACCTCGTGT AG 12

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATTCGGGTA CA 12

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCCTTAGTG AA 12

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCAGTTATGA CC 12

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCAGCTATCT CT 12

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGAAGGCAGT TG 12

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGACAGAGCA TA 12

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGTTTCGCTT CA 12

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGCTGTTTGG TTTGCTCTAG CCCAGTG 27

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CAACTTTCCA CATGGCGCGA TTATTTG 27

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGGAACTGC CGAAGATCGT ACAGCA 26

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO - 647 - PREMARKER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGACAGAGCA TAGTTGGATG GAAACAATCC GATCAGCGGG AACGTTTTTG ATAGATTCTG 60

CTGTTTGGTT TGCTCTAGCC CAGTGCCACG TGTCACAAAT AAGTTGCGCA TTATCTCGGC 120

CGCATGCTTC TGCTACTCGC CAAGCTGCTT GTAAGTCTGC TACACCGCTA TATGGCATAA 180

ATTCTAAACC AATAATTAAT TCTTCTGCGC GGTCACATAA TTCACCAAGC GCGACGATGA 240

TTTGTTCCTC AGGGATTTTT TCAAGCAAAC CACAATTAAT ATGTTTGACG CCAAATAATC 300

GCGCCATGTG GAAAGTTGTT TGCTCTTTCT TTTGTTGTTC TGCTGTACGA TCTTCGGCAG 360

TTCCCCACTG AGTTATGTAC TCCACTTCTG TTACTTTCAT GTTATGCTCT GTCC 414

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO - 647 - PREMARKER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGACAGAGCA TAACATGAAA GTAACAGAAG TGGAGTACAT AACTCAGTGG GGAACTGCCG 60

AAGATCGTAC AGCAGAACAA CAAAAGAAAG AGCAAACAAC TTTCCACATG GCGCGATTAT 120

TTGGCGTCAA ACATATTAAT TGTGGTTTGC TTGAAAAAAT CCCTGAGGAA CAAATCATCG 180

TCGCGCTTGG TGAATTATGT GACCGCGCAG AAGAATTAAT TATTGGTTTA GAATTTATGC 240

CATATAGCGG TGTAGCAGAC TTACAAGCAG CTTGGCGAGT AGCAGAAGCA TGCGGCCGAG 300

ATAATGCGCA ACTTATTTGT GACACGTGGC ACTGGGCTAG AGCAAACCAA ACAGCAGAAT 360

CTATCAAAAA CGTTCCCGCT GATCGGATTG TTTCCATCCA ACTATGCTCT GTCC 414

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: L MONO - 1324 - PREMARKER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGACAGAGCA TAGTTGGATA GAAACAATCC GATCAGCGGG AACATTTTTG ATAGATTCAG 60

CTGTTTGATT TGCTCTAGCC CAGTGCCATG TGTCACAAAT AAGTTGCGCG TTATCTCGTC 120

CGCATGCTTC TGCTACTCGC CAAGCTGCTT GTAAGTCTGC TACACCGCTA TATGGCATAA 180

ATTCTAAACC AATAATTAAT TCTTCTGCGC GGTCACATAA TTCACCAAGC GCGACAATGA 240

TTTGTTCCTC AGGGATTTTT TCAAGCAAAC CACAATTAAT ATGTTTGACG CCAAATAATC 300

GCGCCATGTG GAAAGTTGTT TGCTCTTTCT TTTGTTGTTC TGCTGTACGA TCTTCGGCAG 360

TTCCCCACTG GGTTATGTAC TCCACTTCTG TTACTTTCAT GTTATGCTCT GTCC 414

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO - 1324 - PREMARKER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGACAGAGCA TAACATGAAA GTAACAGAAG TGGAGTACAT AACCCAGTGG GGAACTGCCG 60

AAGATCGTAC AGCAGAACAA CAAAAGAAAG AGCAAACAAC TTTCCACATG GCGCGATTAT 120

TTGGCGTCAA ACATATTAAT TGTGGTTTGC TTGAAAAAAT CCCTGAGGAA CAAATCATTG 180

TCGCGCTTGG TGAATTATGT GACCGCGCAG AAGAATTAAT TATTGGTTTA GAATTTATGC 240

CATATAGCGG TGTAGCAGAC TTACAAGCAG CTTGGCGAGT AGCAGAAGCA TGCGGACGAG 300

ATAACGCGCA ACTTATTTGT GACACATGGC ACTGGGCTAG AGCAAATCAA ACAGCTGAAT 360

CTATCAAAAA TGTTCCCGCT GATCGGATTG TTTCTATCCA ACTATGCTCT GTCC 414

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO - 647 - D. FRAG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TAAGCAAGGA AGTATCTGAT AAAGTCCATC TGTATTTGCA TAGTTGTTAC ACATTGGCGA 60

TACGACAAAT CTGTTAGGCA CCTTCATCGG CCCGATATCA ATAGGTGAAA ACATCGAATT 120

AAATTTCAAA AATAACACAT TCCTTTCACA GGGAGTCTTC CTACTACGTT ATTATTTTCA 180

CATAAGCGAG TAGGTGTTTA GCGTGGAGAA ATTTCTGGCC ATGCTTCGTC TAATACTTTT 240

TTCGTGGCAT TGTATACTTT AAGGGCGGCA TACTCTAAAC CAGTTGCTAC CATAGAGTCT 300

GAAATAACTT CGACTCCCAT CACACGTGGA TTTACACCAT GTTCTTTTAA AATTTTTGCA 360

AAACCGACCG TATCTCCGTA GCCTTCTCCA GGAGCTAGAC GATCATGAAG TGATTCCTCA 420

CGAAGTTCTT TGTAAGGCGT TTCGTGGACA TCGCATAGTT GGATGGAAAC AATCCGATCA 480

GCGGGAACGT TTTTGATAGA TTCTGCTGTT TGGTTTGCTC TAGCCCAGTG CCACGTGTCA 540

CAAATAAGTT GCGCATTATC TCGGCCGCAT GCTTCTGCTA CTCGCCAAGC TGCTTGTAAG 600

TCTGCTACAC CGCTATATGG CATAAATTCT AAACCAATAA TTAATTCTTC TGCGCGGTCA 660

CATAATTCAC CAAGCGCGAC GATGATTTGT TCCTCAGGGA TTTTTTCAAG CAAACCACAA 720

TTAATATGTT TGACGCCAAA TAATCGCGCC ATGTGGAAAG TTGTTTGCTC TTTCTTTTGT 780

TGTTCTGCTG TACGATCTTC GGCAGTTCCC CACTGAGTTA TGTACTCCAC TTCTGTTACT 840

TTCATGTTAT GCTCGTCTAA AATCCGCAAC ATGTCTTCAT CCGTTAATCC GGCAGCTAGT 900

GCATCTACAT AGTTTTCTGC ACGCAAGCCA ATTCCGTCAA AACCGTTTTC CGCAGCGACT 960

TTTACTCGTT TAGGAAAAGA TACCTCTGTT CCAAGCGTAT AAGAGCTAAT CGTGATGGGG 1020

CATTTTTTTA GGTTGCCATT TGCATTTGTC ATAAAAATTA TCTCCTCTCC ATAATAAAAA 1080

TTACAAGAAA CTTTGATAAT ATTTTCACAA ACACCAGTAA AAAAATTAAT TCCGCTTAAT 1140

TAAAAACCTC TGATGTGATA ACGCCTTCAA TAGTTGAAAA TGGAACTGGA CAGTTAACCT 1200

ATTCTACCGT ATATTGGTTT TTAAGGAATA GTTTATTTCA CTGGCGTAAC TACAGTCTAA 1260

TTGTATTATG ACTATTCCAT AAAAACAAAT TGGTATTGTT CTATTAATTG ATAGATAAAT 1320

TGCATAGATA ACTTTTTAGT TAGGAGAGAA GCAT 1354

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 647 - D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATGCTTCTCT CCTAACTAAA AAGTTATCTA TGCAATTTAT CTATCAATTA ATAGAACAAT 60

ACCAATTTGT TTTTATGGAA TAGTCATAAT ACAATTAGAC TGTAGTTACG CCAGTGAAAT 120

AAACTATTCC TTAAAAACCA ATATACGGTA GAATAGGTTA ACTGTCCAGT TCCATTTTCA 180

ACTATTGAAG GCGTTATCAC ATCAGAGGTT TTTAATTAAG CGGAATTAAT TTTTTTACTG 240

GTGTTTGTGA AAATATTATC AAAGTTTCTT GTAATTTTTA TTATGGAGAG GAGATAATTT 300

TTATGACAAA TGCAAATGGC AACCTAAAAA AATGCCCCAT CACGATTAGC TCTTATACGC 360

TTGGAACAGA GGTATCTTTT CCTAAACGAG TAAAAGTCGC TGCGGAAAAC GGTTTTGACG 420

GAATTGGCTT GCGTGCAGAA AACTATGTAG ATGCACTAGC TGCCGGATTA ACGGATGAAG 480

ACATGTTGCG GATTTTAGAC GAGCATAACA TGAAAGTAAC AGAAGTGGAG TACATAACTC 540

AGTGGGGAAC TGCCGAAGAT CGTACAGCAG AACAACAAAA GAAAGAGCAA ACAACTTTCC 600

ACATGGCGCG ATTATTTGGC GTCAAACATA TTAATTGTGG TTTGCTTGAA AAAATCCCTG 660

AGGAACAAAT CATCGTCGCG CTTGGTGAAT TATGTGACCG CGCAGAAGAA TTAATTATTG 720

GTTTAGAATT TATGCCATAT AGCGGTGTAG CAGACTTACA AGCAGCTTGG CGAGTAGCAG 780

AAGCATGCGG CCGAGATAAT GCGCAACTTA TTTGTGACAC GTGGCACTGG GCTAGAGCAA 840

ACCAAACAGC AGAATCTATC AAAAACGTTC CCGCTGATCG GATTGTTTCC ATCCAACTAT 900

GCGATGTCCA CGAAACGCCT TACAAAGAAC TTCGTGAGGA ATCACTTCAT GATCGTCTAG 960

CTCCTGGAGA AGGCTACGGA GATACGGTCG GTTTTGCAAA AATTTTAAAA GAACATGGTG 1020

TAAATCCACG TGTGATGGGA GTCGAAGTTA TTTCAGACTC TATGGTAGCA ACTGGTTTAG 1080

AGTATGCCGC CCTTAAAGTA TACAATGCCA CGAAAAAAGT ATTAGACGAA GCATGGCCAG 1140

AAATTTCTCC ACGCTAAACA CCTACTCGCT TATGTGAAAA TAATAACGTA GTAGGAAGAC 1200

TCCCTGTGAA AGGAATGTGT TATTTTTGAA ATTTAATTCG ATGTTTTCAC CTATTGATAT 1260

CGGGCCGATG AAGGTGCCTA ACAGATTTGT CGTATCGCCA ATGTGTAACA ACTATGCAAA 1320

TACAGATGGA CTTTATCAGA TACTTCCTTG CTTA 1354

2. INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 899 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATCTGTATTT GCATAGTTGT TACACATTGG CGATACGACA AATCTGTTAG GCACCTTCAT 60

CGGCCCGATA TCAATAGGTG AAAACATCGA ATTAAATTTC AAAAATAACA CATTCCTTTC 120

ACAGGGAGTC TTCCTACTAC GTTATTATTT TCACATAAGC GAGTAGGTGT TTAGCGTGGA 180

GAAATTTCTG GCCATGCTTC GTCTAATACT TTTTTCGTGG CATTGTATAC TTTAAGGGCG 240

GCATACTCTA AACCAGTTGC TACCATAGAG TCTGAAATAA CTTCGACTCC CATCACACGT 300

GGATTTACAC CATGTTCTTT TAAAATTTTT GCAAAACCGA CCGTATCTCC GTAGCCTTCT 360

CCAGGAGCTA GACGATCATG AAGTGATTCC TCACGAAGTT CTTTGTAAGG CGTTTCGTGG 420

ACATCGCATA GTTGGATGGA AACAATCCGA TCAGCGGGAA CGTTTTTGAT AGATTCTGCT 480

GTTTGGTTTG CTCTAGCCCA GTGCCACGTG TCACAAATAA GTTGCGCATT ATCTCGGCCG 540

CATGCTTCTG CTACTCGCCA AGCTGCTTGT AAGTCTGCTA CACCGCTATA TGGCATAAAT 600

TCTAAACCAA TAATTAATTC TTCTGCGCGG TCACATAATT CACCAAGCGC GACGATGATT 660

TGTTCCTCAG GGATTTTTTC AAGCAAACCA CAATTAATAT GTTTGACGCC AAATAATCGC 720

GCCATGTGGA AAGTTGTTTG CTCTTTCTTT TGTTGTTCTG CTGTACGATC TTCGGCAGTT 780

CCCCACTGAG TTATGTACTC CACTTCTGTT ACTTTCATGT TATGCTCGTC TAAAATCCGC 840

AACATGTCTT CATCCGTTAA TCCGGCAGCT AGTGCATCTA CATAGTTTTC TGCACGCAAG 900

CCAATTCCGT CAAAACCGTT TTCCGCAGCG ACTTTTACTC GTTTAGGAAA AGATACCTCT 960

GTTCCAAGCG TATAAGAGCT AATCGTGATG GGGCATTTTT TTAGGTTGCC ATTTGCATTT 1020

GTCATAAAAA TTATCTCCTC TCCATAATAA AAATTACAAG AAACTTTGAT AATATTTTCA 1080

CAAACACCAG TAAAAAAATT AATTCCGCTT AATTAAAAAC CTCTGATGTG ATAACGCCTT 1140

CAATAGTTGA AAATGGAACT GGACAGTTAA CCTATTCTAC CGTATATTGG TTTTTAAGGA 1200

ATAGTTTATT TCACTGGCGT AACTACAGTC TAATTGTATT ATGACTATTC CATAAAAACA 1260

AATTGGTATT GTTCTATTAA TTGATAGATA AATTGCATAG ATAACTTTTT AGTTAGGAGA 1320

GAAGCAT 1327

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 899 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGCTTCTCT CCTAACTAAA AAGTTATCTA TGCAATTTAT CTATCAATTA ATAGAACAAT 60

ACCAATTTGT TTTTATGGAA TAGTCATAAT ACAATTAGAC TGTAGTTACG CCAGTGAAAT 120

AAACTATTCC TTAAAAACCA ATATACGGTA GAATAGGTTA ACTGTCCAGT TCCATTTTCA 180

ACTATTGAAG GCGTTATCAC ATCAGAGGTT TTTAATTAAG CGGAATTAAT TTTTTTACTG 240

GTGTTTGTGA AAATATTATC AAAGTTTCTT GTAATTTTTA TTATGGAGAG GAGATAATTT 300

TTATGACAAA TGCAAATGGC AACCTAAAAA AATGCCCCAT CACGATTAGC TCTTATACGC 360

TTGGAACAGA GGTATCTTTT CCTAAACGAG TAAAAGTCGC TGCGGAAAAC GGTTTTGACG 420

GAATTGGCTT GCGTGCAGAA AACTATGTAG ATGCACTAGC TGCCGGATTA ACGGATGAAG 480

ACATGTTGCG GATTTTAGAC GAGCATAACA TGAAAGTAAC AGAAGTGGAG TACATAACTC 540

AGTGGGGAAC TGCCGAAGAT CGTACAGCAG AACAACAAAA GAAAGAGCAA ACAACTTTCC 600

ACATGGCGCG ATTATTTGGC GTCAAACATA TTAATTGTGG TTTGCTTGAA AAAATCCCTG 660

AGGAACAAAT CATCGTCGCG CTTGGTGAAT TATGTGACCG CGCAGAAGAA TTAATTATTG 720

GTTTAGAATT TATGCCATAT AGCGGTGTAG CAGACTTACA AGCAGCTTGG CGAGTAGCAG 780

AAGCATGCGG CCGAGATAAT GCGCAACTTA TTTGTGACAC GTGGCACTGG GCTAGAGCAA 840

ACCAAACAGC AGAATCTATC AAAAACGTTC CCGCTGATCG GATTGTTTCC ATCCAACTAT 900

GCGATGTCCA CGAAACGCCT TACAAAGAAC TTCGTGAGGA ATCACTTCAT GATCGTCTAG 960

CTCCTGGAGA AGGCTACGGA GATACGGTCG GTTTTGCAAA AATTTTAAAA GAACATGGTG 1020

TAAATCCACG TGTGATGGGA GTCGAAGTTA TTTCAGACTC TATGGTAGCA ACTGGTTTAG 1080

AGTATGCCGC CCTTAAAGTA TACAATGCCA CGAAAAAAGT ATTAGACGAA GCATGGCCAG 1140

AAATTTCTCC ACGCTAAACA CCTACTCGCT TATGTGAAAA TAATAACGTA GTAGGAAGAC 1200

TCCCTGTGAA AGGAATGTGT TATTTTTGAA ATTTAATTCG ATGTTTTCAC CTATTGATAT 1260

CGGGCCGATG AAGGTGCCTA ACAGATTTGT CGTATCGCCA ATGTGTAACA ACTATGCAAA 1320

TACAGAT 1327

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1274 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 3386 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGGCACCTTC ATCGGCCCGA TATCAATAGG TGAAAACATC GAATTAAATT TCAAAAATAA 60

CACATTCCTT TCACAGGGAG TCTTCCTACT ACGTTATTAT TTTCACATAA GCGAGTAGGT 120

GTTTAGCGTG GAGAAATTTC TGGCCATGCT TCGTCTAATA CTTTTTTCGT GGCATTGTAT 180

ACTTTAAGGG CGGCATACTC TAAACCAGTT GCTACCATAG AGTCTGAAAT AACTTCGACT 240

CCCATCACAC GTGGATTTAC ACCATGTTCT TTTAAAATTT TTGCAAAACC GACCGTATCT 300

CCGTAGCCTT CTCCAGGAGC TAGACGATCA TGAAGTGATT CCTCACGAAG TTCTTTGTAA 360

GGCGTTTCGT GGACATCGCA TAGTTGGATG GAAACAATCC GATCAGCGGG AACGTTTTTG 420

ATAGATTCTG CTGTTTGGTT TGCTCTAGCC CAGTGCCACG TGTCACAAAT AAGTTGCGCA 480

TTATCTCGGC CGCATGCTTC TGCTACTCGC CAAGCTGCTT GTAAGTCTGC TACACCGCTA 540

TATGGCATAA ATTCTAAACC AATAATTAAT TCTTCTGCGC GGTCACATAA TTCACCAAGC 600

GCGACGATGA TTTGTTCCTC AGGGATTTTT TCAAGCAAAC CACAATTAAT ATGTTTGACG 660

CCAAATAATC GCGCCATGTG GAAAGTTGTT TGCTCTTTCT TTTGTTGTTC TGCTGTACGA 720

TCTTCGGCAG TTCCCCACTG AGTTATGTAC TCCACTTCTG TTACTTTCAT GTTATGCTCG 780

TCTAAAATCC GCAACATGTC TTCATCCGTT AATCCGGCAG CTAGTGCATC TACATAGTTT 840

TCTGCACGCA AGCCAATTCC GTCAAAACCG TTTTCCGCAG CGACTTTTAC TCGTTTAGGA 900

AAAGATACCT CTGTTCCAAG CGTATAAGAG CTAATCGTGA TGGGGCATTT TTTTAGGTTG 960

CCATTTGCAT TTGTCATAAA AATTATCTCC TCTCCATAAT AAAAATTACA AGAAACTTTG 1020

ATAATATTTT CACAAACACC AGTAAAAAAA TTAATTCCGC TTAATTAAAA ACCTCTGATG 1080

TGATAACGCC TTCAATAGTT GAAAATGGAA CTGGACAGTT AACCTATTCT ACCGTATATT 1140

GGTTTTTAAG GAATAGTTTA TTTCACTGGC GTAACTACAG TCTAATTGTA TTATGACTAT 1200

TCCATAAAAA CAAATTGGTA TTGTTCTATT AATTGATAGA TAAATTGCAT AGATAACTTT 1260

TTAGTTAGGA GAGA 1274

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1274 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 3386 D.F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TCTCTCCTAA CTAAAAAGTT ATCTATGCAA TTTATCTATC AATTAATAGA ACAATACCAA 60

TTTGTTTTTA TGGAATAGTC ATAATACAAT TAGACTGTAG TTACGCCAGT GAAATAAACT 120

ATTCCTTAAA AACCAATATA CGGTAGAATA GGTTAACTGT CCAGTTCCAT TTTCAACTAT 180

TGAAGGCGTT ATCACATCAG AGGTTTTTAA TTAAGCGGAA TTAATTTTTT TACTGGTGTT 240

TGTGAAAATA TTATCAAAGT TTCTTGTAAT TTTTATTATG GAGAGGAGAT AATTTTTATG 300

ACAAATGCAA ATGGCAACCT AAAAAAATGC CCCATCACGA TTAGCTCTTA TACGCTTGGA 360

ACAGAGGTAT CTTTTCCTAA ACGAGTAAAA GTCGCTGCGG AAAACGGTTT TGACGGAATT 420

GGCTTGCGTG CAGAAAACTA TGTAGATGCA CTAGCTGCCG GATTAACGGA TGAAGACATG 480

TTGCGGATTT TAGACGAGCA TAACATGAAA GTAACAGAAG TGGAGTACAT AACTCAGTGG 540

GGAACTGCCG AAGATCGTAC AGCAGAACAA CAAAAGAAAG AGCAAACAAC TTTCCACATG 600

GCGCGATTAT TTGGCGTCAA ACATATTAAT TGTGGTTTGC TTGAAAAAAT CCCTGAGGAA 660

CAAATCATCG TCGCGCTTGG TGAATTATGT GACCGCGCAG AAGAATTAAT TATTGGTTTA 720

GAATTTATGC CATATAGCGG TGTAGCAGAC TTACAAGCAG CTTGGCGAGT AGCAGAAGCA 780

TGCGGCCGAG ATAATGCGCA ACTTATTTGT GACACGTGGC ACTGGGCTAG AGCAAACCAA 840

ACAGCAGAAT CTATCAAAAA CGTTCCCGCT GATCGGATTG TTTCCATCCA ACTATGCGAT 900

GTCCACGAAA CGCCTTACAA AGAACTTCGT GAGGAATCAC TTCATGATCG TCTAGCTCCT 960

GGAGAAGGCT ACGGAGATAC GGTCGGTTTT GCAAAAATTT TAAAAGAACA TGGTGTAAAT 1020

CCACGTGTGA TGGGAGTCGA AGTTATTTCA GACTCTATGG TAGCAACTGG TTTAGAGTAT 1080

GCCGCCCTTA AAGTATACAA TGCCACGAAA AAAGTATTAG ACGAAGCATG GCCAGAAATT 1140

TCTCCACGCT AAACACCTAC TCGCTTATGT GAAAATAATA ACGTAGTAGG AAGACTCCCT 1200

GTGAAAGGAA TGTGTTATTT TTGAAATTTA ATTCGATGTT TTCACCTATT GATATCGGGC 1260

CGATGAAGGT GCCT 1274

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1289 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 1324 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAAATCTGTT AGGCACCTTC ATCGGCCCGA TATCAATAGG TGAAAACATC GAATTAAATT 60

TCAAAAATAA CACATTCCTT TCACAGGGAG TCTTCCTACT ACGTTATCAT TTTCACATAA 120

CTAAGTAGGT GTTTAGCGTG GAGAAATCTC TGGCCATGCT TCGTCTAATA CTTTTTTCGT 180

GGCATTGTAT ACTTTAAGAG CGGCATACTC TAAACCAGTT GCTACCATAG AGTCTGAAAT 240

AACTTCAACT CCCATCACAC GTGGATTTAC ACCATGCTCT TTTAAAATTT TTGCAAAACC 300

GACCGTATCT CCGTATCCTT CTCCAGGAGC TAAACGATCA TGAAGTGATT CTTCACGAAG 360

TTCTTTGTAA GGTGTTTCGT GGACATCGCA TAGTTGGATA GAAACAATCC GATCAGCGGG 420

AACATTTTTG ATAGATTCAG CTGTTTGATT TGCTCTAGCC CAGTGCCATG TGTCACAAAT 480

AAGTTGCGCG TTATCTCGTC CGCATGCTTC TGCTACTCGC CAAGCTGCTT GTAAGTCTGC 540

TACACCGCTA TATGGCATAA ATTCTAAACC AATAATTAAT TCTTCTGCGC GGTCACATAA 600

TTCACCAAGC GCGACAATGA TTTGTTCCTC AGGGATTTTT TCAAGCAAAC CACAATTAAT 660

ATGTTTGACG CCAAATAATC GCGCCATGTG GAAAGTTGTT TGCTCTTTCT TTTGTTGTTC 720

TGCTGTACGA TCTTCGGCAG TTCCCCACTG GGTTATGTAC TCCACTTCTG TTACTTTCAT 780

GTTATGCTCG TCTAAAATCC GCAACATGTC TTCATCGGTT AATCCGGCAG CTAGTGCATC 840

TACATAATTT TCTGCACGCA AGCCAATTCC GTCAAAACCA TTTTCCGCAG CGACTTTCAC 900

TCGTTTAGGA AAAGATACCT CCGTTCCTAG TGTGTAAGAG CTAATCGTGA TGGGGCATTT 960

TTTTAGATTG CCATTTGCAT TTGTCATAAA AATTATCTCC TCTCCATAAT AAAAATTACA 1020

AGAAACTTTG ATAATATTTT CACAAACACC AGTAAAAAAA TAAATTCCAC TAAATTAAAA 1080

ATCTCTGATG TGATAACGCC TTCAATAGTT AAAAATGGAA CTGGACAGTT AACCTATTCT 1140

ACCGTATATT GGTTTTTAAG GAATAGTTTA TTTCACTGGC GTAACTACAG TTTAATTGTA 1200

TTATGACTAT TCCATAAAAA CAAATTGGTA TTGTTCTATT AATTGATAGA TAAATTGCAT 1260

AGATAACTTT TTAGTTAGGA GAGAAGCAT 1289

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1289 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 1324 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ATGCTTCTCT CCTAACTAAA AAGTTATCTA TGCAATTTAT CTATCAATTA ATAGAACAAT 60

ACCAATTTGT TTTTATGGAA TAGTCATAAT ACAATTAAAC TGTAGTTACG CCAGTGAAAT 120

AAACTATTCC TTAAAAACCA ATATACGGTA GAATAGGTTA ACTGTCCAGT TCCATTTTTA 180

ACTATTGAAG GCGTTATCAC ATCAGAGATT TTTAATTTAG TGGAATTTAT TTTTTTACTG 240

GTGTTTGTGA AAATATTATC AAAGTTTCTT GTAATTTTTA TTATGGAGAG GAGATAATTT 300

TTATGACAAA TGCAAATGGC AATCTAAAAA AATGCCCCAT CACGATTAGC TCTTACACAC 360

TAGGAACGGA GGTATCTTTT CCTAAACGAG TGAAAGTCGC TGCGGAAAAT GGTTTTGACG 420

GAATTGGCTT GCGTGCAGAA AATTATGTAG ATGCACTAGC TGCCGGATTA ACCGATGAAG 480

ACATGTTGCG GATTTTAGAC GAGCATAACA TGAAAGTAAC AGAAGTGGAG TACATAACCC 540

AGTGGGGAAC TGCCGAAGAT CGTACAGCAG AACAACAAAA GAAAGAGCAA ACAACTTTCC 600

ACATGGCGCG ATTATTTGGC GTCAAACATA TTAATTGTGG TTTGCTTGAA AAAATCCCTG 660

AGGAACAAAT CATTGTCGCG CTTGGTGAAT TATGTGACCG CGCAGAAGAA TTAATTATTG 720

GTTTAGAATT TATGCCATAT AGCGGTGTAG CAGACTTACA AGCAGCTTGG CGAGTAGCAG 780

AAGCATGCGG ACGAGATAAC GCGCAACTTA TTTGTGACAC ATGGCACTGG GCTAGAGCAA 840

ATCAAACAGC TGAATCTATC AAAAATGTTC CCGCTGATCG GATTGTTTCT ATCCAACTAT 900

GCGATGTCCA CGAAACACCT TACAAAGAAC TTCGTGAAGA ATCACTTCAT GATCGTTTAG 960

CTCCTGGAGA AGGATACGGA GATACGGTCG GTTTTGCAAA AATTTTAAAA GAGCATGGTG 1020

TAAATCCACG TGTGATGGGA GTTGAAGTTA TTTCAGACTC TATGGTAGCA ACTGGTTTAG 1080

AGTATGCCGC TCTTAAAGTA TACAATGCCA CGAAAAAAGT ATTAGACGAA GCATGGCCAG 1140

AGATTTCTCC ACGCTAAACA CCTACTTAGT TATGTGAAAA TGATAACGTA GTAGGAAGAC 1200

TCCCTGTGAA AGGAATGTGT TATTTTTGAA ATTTAATTCG ATGTTTTCAC CTATTGATAT 1260

CGGGCCGATG AAGGTGCCTA ACAGATTTG 1289

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO ORF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Thr Asn Ala Asn Gly Asn Leu Lys Lys Cys Pro Ile Thr Ile Ser
1               5                  10                  15

Ser Tyr Thr Leu Gly Thr Glu Val Ser Phe Pro Lys Arg Val Lys Val
            20                  25                  30

Ala Ala Glu Asn Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr
        35                  40                  45

Val Asp Ala Leu Ala Ala Gly Leu Thr Asp Glu Asp Met Leu Arg Ile
    50                  55                  60

Leu Asp Glu His Asn Met Lys Val Thr Glu Val Glu Tyr Ile Thr Gln
65                  70                  75                  80

Trp Gly Thr Ala Glu Asp Arg Thr Ala Glu Gln Gln Lys Lys Glu Gln
                85                  90                  95

Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys His Ile Asn Cys
            100                 105                 110

Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Val Ala Leu Gly
        115                 120                 125

Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met
    130                 135                 140

Pro Tyr Ser Gly Val Ala Asp Leu Gln Ala Ala Trp Arg Val Ala Glu
145                 150                 155                 160

Ala Cys Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp
                165                 170                 175

Ala Arg Ala Asn Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp
            180                 185                 190

Arg Ile Val Ser Ile Gln Leu Cys Asp Val His Glu Thr Pro Tyr Lys
        195                 200                 205

Glu Leu Arg Glu Glu Ser Leu His Asp Arg Leu Ala Pro Gly Glu Gly
    210                 215                 220

Tyr Gly Asp Thr Val Gly Phe Ala Lys Ile Leu Lys Glu His Gly Val
225                 230                 235                 240

Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp Ser Met Val Ala
                245                 250                 255

Thr Gly Leu Glu Tyr Ala Ala Leu Lys Val Tyr Asn Ala Thr Lys Lys
            260                 265                 270

Val Leu Asp Glu Ala Trp Pro Glu Ile Ser Pro Arg
        275                 280
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1329 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L INNOCUA 4450 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CATCTGTATT CGCATAGTTA TTACACATTG GAGATACAAC AAAACGATTT GGTACTCTCA 60

TAGGCCCGAT ATCAATCGGT GAGAACATCG AATTAAATTT CAAAAAAAAC ACTTCCCTTT 120

CAAGGTAGAA TCTTCTTGTT ACGTTACTTT TTTCACATGT TTAATTATTT TTTTATTTGG 180

GAGAAATTTC TGGCCATGCT TCGTCCAATA CTTTTTTCGT TGCATTATAT ACTTTGATGG 240

CAGCATATTC TAAACCAGTT TCTACCATGG AGTCGGATAT AACTTCAACG CCCATAACAC 300

GGGGGTTAAC GCCATGCTCT TTCAAGATTC GAGCAAAACC GACTGTATCG CCGTATCCCT 360

CGCCAGGAGC AAGTCGGTCA TGGAGAGATT CTTCCCGAAG TTCTTTGTAA GGTGTTTCGT 420

GTACGTCACA TAATTGGATG GAAACAATCC GGTCAGCGGG AACATTTTTG ATTGATTCTG 480

CTGTTTGGTT TGCTCTAGCC GAGTGCCAAG TGTCGCAAAT TAGTTGTGCA TTATCCCTGC 540

CACATGCTTC AGCTACACGC CAAGCTGCTG CTAAGTCTGC TACACCACTG TATGGCATAA 600

ACTCTAAACC GATAATTAAT TCTTCCGCAC GGTCACACAA TTCACCGAGT GCCGTAATGA 660

TTTGTTCTTC GGGGATTTTT TCAAGCAAAC CGCAGTTAAT ATGTTTGACG CCGAATAAAC 720

GCGCCATGTG GAAAGTAGTT TGTTCTTTCT TTTGCTGTTC GGCTGTGCGG TCCTCGGCGG 780

TTCCCCACTG AGTTATGTAT TCTACTTCTG TTACTTTGAT GTTATGCTCA TCTAAAATCC 840

GCAACATATC TTCATCAGTT AATCCAGCGG CTAGTGCGTC TACATAGTTT TCTGCACGTA 900

AGCCAATTCC ATCAAAACCA TTTTCTGCTG CGATTCTCAC TCGTTCAGGA AAAGATACCT 960

CCGTTCCAAG CGTGTAAGAG CTGATCGTGA TTGGGCATTT TTTTAGGTCG CCATTTGCAT 1020

TTGTCATAAA AATTATCTCC TCTCTAGAAT AAAAATTACA AGAAACTTTG ATAATATTTT 1080

CACAAACACC AGTAAAAAAA TAAATTCCCG TTCATTAAAT ATCGCTGATG TGATAACGCC 1140

TTCAATGTTT GAAATTTCAA CTGGACAGTT AACGTATTCT ACCGTATATT GGTTTTTAAG 1200

GAATAGTTTG TTCTGCTGGT GTAACTACAG TCTAATTGTA TTATGACTAT TCCATAAAAA 1260

CAAATTGGTA TTATTCTATT AATTGATAGA TAAATTGCAT AGATAATTTT TAGTAAGGAG 1320

AGAAGCCAT 1329

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1329 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L INNOCUA 4450 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ATGGCTTCTC TCCTTACTAA AAATTATCTA TGCAATTTAT CTATCAATTA ATAGAATAAT 60

ACCAATTTGT TTTTATGGAA TAGTCATAAT ACAATTAGAC TGTAGTTACA CCAGCAGAAC 120

AAACTATTCC TTAAAAACCA ATATACGGTA GAATACGTTA ACTGTCCAGT TGAAATTTCA 180

AACATTGAAG GCGTTATCAC ATCAGCGATA TTTAATGAAC GGGAATTTAT TTTTTTACTG 240

GTGTTTGTGA AAATATTATC AAAGTTTCTT GTAATTTTTA TTCTAGAGAG GAGATAATTT 300

TTATGACAAA TGCAAATGGC GACCTAAAAA AATGCCCAAT CACGATCAGC TCTTACACGC 360

TTGGAACGGA GGTATCTTTT CCTGAACGAG TGAGAATCGC AGCAGAAAAT GGTTTTGATG 420

GAATTGGCTT ACGTGCAGAA AACTATGTAG ACGCACTAGC CGCTGGATTA ACTGATGAAG 480

ATATGTTGCG GATTTTAGAT GAGCATAACA TCAAAGTAAC AGAAGTAGAA TACATAACTC 540

AGTGGGGAAC CGCCGAGGAC CGCACAGCCG AACAGCAAAA GAAAGAACAA ACTACTTTCC 600

ACATGGCGCG TTTATTCGGC GTCAAACATA TTAACTGCGG TTTGCTTGAA AAAATCCCCG 660

AAGAACAAAT CATTACGGCA CTCGGTGAAT TGTGTGACCG TGCGGAAGAA TTAATTATCG 720

GTTTAGAGTT TATGCCATAC AGTGGTGTAG CAGACTTAGC AGCAGCTTGG CGTGTAGCTG 780

AAGCATGTGG CAGGGATAAT GCACAACTAA TTTGCGACAC TTGGCACTCG GCTAGAGCAA 840

ACCAAACAGC AGAATCAATC AAAAATGTTC CCGCTGACCG GATTGTTTCC ATCCAATTAT 900

GTGACGTACA CGAAACACCT TACAAAGAAC TTCGGGAAGA ATCTCTCCAT GACCGACTTG 960

CTCCTGGCGA GGGATACGGC GATACAGTCG GTTTTGCTCG AATCTTGAAA GAGCATGGCG 1020

TTAACCCCCG TGTTATGGGC GTTGAAGTTA TATCCGACTC CATGGTAGAA ACTGGTTTAG 1080

AATATGCTGC CATCAAAGTA TATAATGCAA CGAAAAAAGT ATTGGACGAA GCATGGCCAG 1140

AAATTTCTCC CAAATAAAAA AATAATTAAA CATGTGAAAA AAGTAACGTA ACAAGAAGAT 1200

TCTACCTTGA AAGGGAAGTG TTTTTTTTGA AATTTAATTC GATGTTCTCA CCGATTGATA 1260

TCGGGCCTAT GAGAGTACCA AATCGTTTTG TTGTATCTCC AATGTGTAAT AACTATGCGA 1320

ATACAGATG 1329

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1319 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L SEELIGERI 3327 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTTGCATA GTTATTACAC ATCGGCGAAA CCACGAAACG GTTTGGCACA CGCATAGGTC 60

CGATATCTAT TGGAGAAAAC ATCGAATTAA ATTTCAAAAA AAACACTTTC CTTTCAAAGG 120

GGGATTTTTA ACGTTATTAA TTTCACATAG CGATGCGTCA AGTTACTTTG GAGAAACTTC 180

TGGCCATGCT TCATCTAATA CTTTTTTAGT AGCATTATAA ACTTTAATTG CCGCATATTC 240

TAAACCTGTT TCTACCATCG AGTCTGAAAT CACTTCCACA CCCATAACAC GCGGATTTAC 300

ACCATGTTCT TTTAAAATAC GAGCAAAGCC TACAGTGTCG CCGTATCCTT CACCAGGTGC 360

TAATCGATCA TGGAGAGATT CTTCACGTAA TTCTTTGTAA GGAGTTTCGT GAACATCGCA 420

AAGTTGAATA GACACAATTC GGTCAGCAGG AATATTTTTG ATAGATTCTG CTGTTTGATT 480

TGCTCTAGCC CAGTGCCAAG TATCACAAAT CAGTTGCGCG TTATCTCGGC CACATGCTTC 540

TGCCACACGC CATGCTGCTG CTAAATCTGC TACACCGCTA TAAGGCATGA ATTCTAAACC 600

GATAATTAAT TCTTCAGCAC GGTCACAAAG TTCACCCAGA GCAGTAATGA TTTGTTCTTC 660

CGGAATCTTT TCAAGTAAAC CACAGTTAAT ATGTTTTACG CCGAATAAGC GCGCCATATG 720

AAAAGTGGTT TGTTCTTTCT TTTGTTGTTC TTTGGTGCGG TCGGAAGCGG TTCCCCATTG 780

CGTTATGTAT TCTACTTCTG TTACTTTGAT GTGATGTTCG TCCAAAATAC GCAACATATC 840

TTCATCGGTT AAGCCTGCTG CAAGTGCATC AACATAGTTT TCTGCACGTA AACCAATTCC 900

ATCAAAACCA TTTTCTGCTG CGATTCGTAC TCGTTCAGGA AAAGAAACCT CCGTTCCAAG 960

CGTGTAAGAA CTAATCGTGA TGGGGCATTT TTTTAAGTCG CCATTTACAT TTGTCATAAA 1020

AATTATCTCC TCTCTAGATT AAAATACAAG AAACTTTGAT AATAATTTCA CAATCACCAG 1080

CAAAAAAATA AATTCCTTTT TAGAATAAAA CGTCCTGAAG TGATAACGCA TTCAATCATT 1140

GAAAATCTGA CTGGACAGTT TTCGAATTCT ACCGTATATT GGTTTTTAAA GGATAGTTTG 1200

TCTCACTGGC TTAATTACAG TTTAATTGTA GTATGACTAT TCCATAAAAA CAAATTGGTA 1260

TTATTCTATT AATTGATAGA TAAATTGCAT AGATGCTTTT TAAAGAGGGG AGAAACCAT 1319

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1319 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L SEELIGERI 3327 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

ATGGTTTCTC CCCTCTTTAA AAAGCATCTA TGCAATTTAT CTATCAATTA ATAGAATAAT 60

ACCAATTTGT TTTTATGGAA TAGTCATACT ACAATTAAAC TGTAATTAAG CCAGTGAGAC 120

AAACTATCCT TTAAAAACCA ATATACGGTA GAATTCGAAA ACTGTCCAGT CAGATTTTCA 180

ATGATTGAAT GCGTTATCAC TTCAGGACGT TTTATTCTAA AAAGGAATTT ATTTTTTTGC 240

TGGTGATTGT GAAATTATTA TCAAAGTTTC TTGTATTTTA ATCTAGAGAG GAGATAATTT 300

TTATGACAAA TGTAAATGGC GACTTAAAAA AATGCCCCAT CACGATTAGT TCTTACACGC 360

TTGGAACGGA GGTTTCTTTT CCTGAACGAG TACGAATCGC AGCAGAAAAT GGTTTTGATG 420

GAATTGGTTT ACGTGCAGAA AACTATGTTG ATGCACTTGC AGCAGGCTTA ACCGATGAAG 480

ATATGTTGCG TATTTTGGAC GAACATCACA TCAAAGTAAC AGAAGTAGAA TACATAACGC 540

AATGGGGAAC CGCTTCCGAC CGCACCAAAG AACAACAAAA GAAAGAACAA ACCACTTTTC 600

ATATGGCGCG CTTATTCGGC GTAAAACATA TTAACTGTGG TTTACTTGAA AAGATTCCGG 660

AAGAACAAAT CATTACTGCT CTGGGTGAAC TTTGTGACCG TGCTGAAGAA TTAATTATCG 720

GTTTAGAATT CATGCCTTAT AGCGGTGTAG CAGATTTAGC AGCAGCATGG CGTGTGGCAG 780

AAGCATGTGG CCGAGATAAC GCGCAACTGA TTTGTGATAC TTGGCACTGG GCTAGAGCAA 840

ATCAAACAGC AGAATCTATC AAAAATATTC CTGCTGACCG AATTGTGTCT ATTCAACTTT 900

GCGATGTTCA CGAAACTCCT TACAAAGAAT TACGTGAAGA ATCTCTCCAT GATCGATTAG 960

CACCTGGTGA AGGATACGGC GACACTGTAG GCTTTGCTCG TATTTTAAAA GAACATGGTG 1020

TAAATCCGCG TGTTATGGGT GTGGAAGTGA TTTCAGACTC GATGGTAGAA ACAGGTTTAG 1080

AATATGCGGC AATTAAAGTT TATAATGCTA CTAAAAAAGT ATTAGATGAA GCATGGCCAG 1140

AAGTTTCTCC AAAGTAACTT GACGCATCGC TATGTGAAAT TAATAACGTT AAAAATCCCC 1200

CTTTGAAAGG AAAGTGTTTT TTTTGAAATT TAATTCGATG TTTTCTCCAA TAGATATCGG 1260

ACCTATGCGT GTGCCAAACC GTTTCGTGGT TTCGCCGATG TGTAATAACT ATGCAAATA 1319

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1304 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L WEISHIMERI 3359 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ATTACACATT GGAGATACAA CAAACCGATT AGGTACTTTC ATCGGGCCAA TATCGATAGG 60

TGAGAACATC GAATTAAATT TCAAAAAAAA CACTTCCCTT TCAAGATGGA ATCTAGATTA 120

CGTTATTATT TTCACATGTT TGAATACATT ATTTTGGAGA AATTTCTGGC CAAGCCTCGT 130

CTAATACTTT TTTCGTGGCA TTATATACTT TAATTGCAGC ATATTCTAGA CCAGTTTCAA 240

CCATAGAGTC AGATATTACT TCGACTCCCA TCACTCGTGG ATTTACACCA TGTTCTTTTA 300

GGATGCGTGC AAAACCAACT GTATCTCCAT ATCCTTCACC AGGTGGTAGT CGGTCATGAA 360

GCGATTCTTC ACGAAGTTCT TTGTATGGCG TTTCATGAAC ATCACACAAT TGGATAGAAA 420

CAATTCGATC AGCTGGGACA TTTTTGATAG ACTCTGCTGT TTGGTTTGCT CTTGCCCAGT 480

GCCAAGTATC GCAAATTAGT TGTGCGTTAT CTCTACCACA TGCTTCTGCA ACACGCCAAG 540

CTGCGGCTAA GTCTGCTACT CCGCTATACG GCATAAATTC TAAACCGATG ATTAATTCTT 600

CGGCACGATC ACATAACTCA CCAAGAGCAG TAATTATTTG CTCTTCTGGA ATTTTTTCAA 660

GTAAACCGCA GTTAATATGT TTTACACCGA ATAACCGAGC CATGTGGAAA GTGGTTTGCT 720

CTTTTTGTTG TTGAGCATCG GTTCGGTCTG CTTCAGTTCC CCACTGAGTT ATGTATTCTA 780

CTTCTGTTAC TTTGATATTA TGCTTGTCTA AAATCTGCAG CATGTCATCA TCAGTTAAGC 840

CAGCTGCAAG AGCGTCTACA TAATTTTCAG CTCGCAAGCC AATTCCGTCA AAACCATTTT 900

CTGCTGCAAT CTTTACACGT TCTGGGAAGG AAACCTCCGT TCCAAGTGTG TAAGAACTAA 960

TCGTGATGGG GCATTTTTTT AAGTTGCCAT TTGAATTTGT CATAAAAATT ATCTCCTCTC 1020

AAGAATGTAA ATTACAAGAA ACTTTGATAA TATTTTCACA AACACCAGTA AAAAAATAAA 1080

TTCCTTTTAA TTAAAAATCG CTGATGTGAT AACGCCTTCA ATGATCAAAA TACAACTGGA 1140

CAGTTAACGT ATTCTACCGT ATATTGGTTT TTAAGGAATA GTTTATTCTG CTGGTGTAAC 1200

TACAGTTTAA TTGTATTATG ACTATTCCAT AAAAACAAAT TGGTATTATT CTATTAATTG 1260

ATAGATAAAT TGCATAGATA CTTTTTAATA AGGGGAGAAG CCAT 1304

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1304 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L WEISHIMERI 3359 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATGGCTTCTC CCCTTATTAA AAAGTATCTA TGCAATTTAT CTATCAATTA ATAGAATAAT 60

ACCAATTTGT TTTTATGGAA TAGTCATAAT ACAATTAAAC TGTAGTTACA CCAGCAGAAT 120

AAACTATTCC TTAAAAACCA ATATACGGTA GAATACGTTA ACTGTCCAGT TGTATTTTGA 180

TCATTGAAGG CGTTATCACA TCAGCGATTT TTAATTAAAA GGAATTTATT TTTTTACTGG 240

TGTTTGTGAA AATATTATCA AAGTTTCTTG TAATTTACAT TCTTGAGAGG AGATAATTTT 300

TATGACAAAT TCAAATGGCA ACTTAAAAAA ATGCCCCATC ACGATTAGTT CTTACACACT 360

TGGAACGGAG GTTTCCTTCC CAGAACGTGT AAAGATTGCA GCAGAAAATG GTTTTGACGG 420

AATTGGCTTG CGAGCTGAAA ATTATGTAGA CGCTCTTGCA GCTGGCTTAA CTGATGATGA 480

CATGCTGCAG ATTTTAGACA AGCATAATAT CAAAGTAACA GAAGTAGAAT ACATAACTCA 540

GTGGGGAACT GAAGCAGACC GAACCGATGC TCAACAACAA AAAGAGCAAA CCACTTTCCA 600

CATGGCTCGG TTATTCGGTG TAAAACATAT TAACTGCGGT TTACTTGAAA AAATTCCAGA 660

AGAGCAAATA ATTACTGCTC TTGGTGAGTT ATGTGATCGT GCCGAAGAAT TAATCATCGG 720

TTTAGAATTT ATGCCGTATA GCGGAGTAGC AGACTTAGCC GCAGCTTGGC GTGTTGCAGA 780

AGCATGTGGT AGAGATAACG CACAACTAAT TTGCGATACT TGGCACTGGG CAAGAGCAAA 840

CCAAACAGCA GAGTCTATCA AAAATGTCCC AGCTGATCGA ATTGTTTCTA TCCAATTGTG 900

TGATGTTCAT GAAACGCCAT ACAAAGAACT TCGTGAAGAA TCGCTTCATG ACCGACTACC 960

ACCTGGTGAA GGATATGGAG ATACAGTTGG TTTTGCACGC ATCCTAAAAG AACATGGTGT 1020

AAATCCACGA GTGATGGGAG TCGAAGTAAT ATCTGACTCT ATGGTTGAAA CTGGTCTAGA 1080

ATATGCTGCA ATTAAAGTAT ATAATGCCAC GAAAAAAGTA TTAGACGAGG CTTGGCCAGA 1140

AATTTCTCCA AAATAATGTA TTCAAACATG TGAAAATAAT AACGTAATCT AGATTCCATC 1200

TTGAAAGGGA AGTGTTTTTT TTGAAATTTA ATTCGATGTT CTCACCTATC GATATTGGCC 1260

CGATGAAAGT ACCTAATCGG TTTGTTGTAT CTCCAATGTG TAAT 1304

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1328 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: L IVANOVII 3340 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CCGTCTGTAT TTGCATAGTT ATTACACATT TGCGAAACAA CAAAACGGTT AGGTACTTTC 60

ATTGGTCCGA TATCTATTGG AGAAAACATC GAATTAAATT TCAAAAAAAA CACTTCCCTT 120

TCAAGATAGA ATTTTTCTTT CGTTATTAAT TTCACATAGT ATTGTATCAG ATTATTTTGG 180

TGACACTTCG GGCCATGCTT CGTCTAATAC TTTTTTTGTG GCATTGTATA CTTTAATTGC 240

GGTATATTCT AAGCCAGTTT CTACCATGGA ATCAGATATT ACTTCTACTC CCATGACACG 300

TGGACTTACA CCATGCTCTT TTAAAATACG AGCAAAACCA ATCGTGTCCC CGTATCCTTC 360

ACCAGGAGCT AGTCTATCAT GCAGTGACTC TTCGCGAAGC TCTTTATAGG GCGTTTCATG 420

GACGTCACAG AGTTGAATTG AAACAATCCG ATCAGCAGGT ACATTTTTGA TAGATTCTGC 480

TGTTTGGTTT GCTCTTGCCC AGTGCCATGT GTCACAAATT AGTTGGGCGT TATCTCTGCC 540

GCAAGCCTCT GCCACACGCC ATGCTGCTGC CAAATCTGCT ACTCCGCTGT AAGGCATGAA 600

TTCTAAACCA ATAATCAATT CTTCTGCACG GTCGCAAAGT TCACCAAGAG CAGTAATGAT 660

TTGGTCTTCG GGGATTTTTT CCAATAAACC ACAATTAATA TGTTTTACAC CGAATAAGCG 720

AGCCATGTGG AAGGTAGTTT GTTCTTTCTT TTGTTGCTCG AAAGTGCGGT CAGAAGCGGT 780

TCCCCACTGC GTTATGTATT CTACTTCAGT AACTTTGATG TGATGCTCAT CTAAAATCCT 840

TAACATATCT TCATCAGTCA AGCCAGCTGC CAGTGCATCG ACATAATTTT CGGCGCGTAA 900

ACCAATTCCG TCAAAACCAT TTTCTGCTGC AATTCGTACT CGTTCAGGAA AAGAAACCTC 960

CGTTCCTAAG GTATAAGAGC TAATCGTGAT GGGGCATTTT TTTAGGTTGC CATTTGCATT 1020

TGTCATAAAA ATTATCTCCT CTCTAGATTA AAACACAAGA AACTTTGATA ATGTTTTCAC 1080

AATCACCAGC AAAAAAATAA AATCCATTCA CTTAGAAAAC TTTCTAATGT GAGAACGCAT 1140

TCAATAGTTA GAAAATTGAC TGGACAGTTT TCACATTCTA CCGTATATTG GTTTTTAAAG 1200

GTTAGTTTAT TTCACTGGCA TAACTACTGT TTAATTGTAG TATGACTATT CCATAAAAAC 1260

AAATTGGTAT TATTCTATTA ATCGATAGAT AAATTGCATA GATTATTTTT AACAAGGAGA 1320

GAACCCAT 1328

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1328 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(B) STRAIN: L IVANOVII 3340 D.F.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ATGGGTTCTC TCCTTGTTAA AAATAATCTA TGCAATTTAT CTATCGATTA ATAGAATAAT 60

ACCAATTTGT TTTTATGGAA TAGTCATACT ACAATTAAAC AGTAGTTATG CCAGTGAAAT 120

AAACTAACCT TTAAAAACCA ATATACGGTA GAATGTGAAA ACTGTCCAGT CAATTTTCTA 180

ACTATTGAAT GCGTTCTCAC ATTAGAAAGT TTTCTAAGTG AATGGATTTT ATTTTTTTGC 240

TGGTGATTGT GAAAACATTA TCAAAGTTTC TTGTGTTTTA ATCTAGAGAG GAGATAATTT 300

TTATGACAAA TGCAAATGGC AACCTAAAAA AATGCCCCAT CACGATTAGC TCTTATACCT 360

TAGGAACGGA GGTTTCTTTT CCTGAACGAG TACGAATTGC AGCAGAAAAT GGTTTTGACG 420

GAATTGGTTT ACGCGCCGAA AATTATGTCG ATGCACTGGC AGCTGGCTTG ACTGATGAAG 480

ATATGTTAAG GATTTTAGAT GAGCATCACA TCAAAGTTAC TGAAGTAGAA TACATAACGC 540

AGTGGGGAAC CGCTTCTGAC CGCACTTTCG AGCAACAAAA GAAAGAACAA ACTACCTTCC 600

ACATGGCTCG CTTATTCGGT GTAAAACATA TTAATTGTGG TTTATTGGAA AAAATCCCCG 660

AAGACCAAAT CATTACTGCT CTTGGTGAAC TTTGCGACCG TGCAGAAGAA TTGATTATTG 720

GTTTAGAATT CATGCCTTAC AGCGGAGTAG CAGATTTGGC AGCAGCATGG CGTGTGGCAG 780

AGGCTTGCGG CAGAGATAAC GCCCAACTAA TTTGTGACAC ATGGCACTGG GCAAGAGCAA 840

ACCAAACAGC AGAATCTATC AAAAATGTAC CTGCTGATCG GATTGTTTCA ATTCAACTCT 900

GTGACGTCCA TGAAACGCCC TATAAAGAGC TTCGCGAAGA GTCACTGCAT GATAGACTAG 960

CTCCTGGTGA AGGATACGGG GACACGATTG GTTTTGCTCG TATTTTAAAA GAGCATGGTG 1020

TAAGTCCACG TGTCATGGGA GTAGAAGTAA TATCTGATTC CATGGTAGAA ACTGGCTTAG 1080

AATATACCGC AATTAAAGTA TACAATGCCA CAAAAAAAGT ATTAGACGAA GCATGGCCCG 1140

AAGTGTCACC AAAATAATCT GATACAATAC TATGTGAAAT TAATAACGAA AGAAAAATTC 1200

TATCTTGAAA GGGAAGTGTT TTTTTTGAAA TTTAATTCGA TGTTTTCTCC AATAGATATC 1260

GGACCAATGA AAGTACCTAA CCGTTTTGTT GTTTCGCAAA TGTGTAATAA CTATGCAAAT 1320

ACAGACGG 1328

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 647 ORF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Thr Asn Ala Asn Gly Asn Leu Lys Lys Cys Pro Ile Thr Ile Ser
1 5 10 15

Ser Tyr Thr Leu Gly Thr Glu Val Ser Phe Pro Lys Arg Val Lys Val
20 25 30

Ala Ala Glu Asn Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr
35 40 45

Val Asp Ala Leu Ala Ala Gly Leu Thr Asp Glu Asp Met Leu Arg Ile
50 55 60

Leu Asp Glu His Asn Met Lys Val Thr Glu Val Glu Tyr Ile Thr Gln
65 70 75 80

```
Trp Gly Thr Ala Glu Asp Arg Thr Ala Glu Gln Gln Lys Lys Glu Gln
                85                  90                  95

Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys His Ile Asn Cys
            100                 105                 110

Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Val Ala Leu Gly
        115                 120                 125

Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met
    130                 135                 140

Pro Tyr Ser Gly Val Ala Asp Leu Gln Ala Ala Trp Arg Val Ala Glu
145                 150                 155                 160

Ala Cys Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp
                165                 170                 175

Ala Arg Ala Asn Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp
            180                 185                 190

Arg Ile Val Ser Ile Gln Leu Cys Asp Val His Glu Thr Pro Tyr Lys
        195                 200                 205

Glu Leu Arg Glu Glu Ser Leu His Asp Arg Leu Ala Pro Gly Glu Gly
    210                 215                 220

Tyr Gly Asp Thr Val Gly Phe Ala Lys Ile Leu Lys Glu His Gly Val
225                 230                 235                 240

Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp Ser Met Val Ala
                245                 250                 255

Thr Gly Leu Glu Tyr Ala Ala Leu Lys Val Tyr Asn Ala Thr Lys Lys
            260                 265                 270

Val Leu Asp Glu Ala Trp Pro Glu Ile Ser Pro Arg
        275                 280
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 4450 ORF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Met Thr Asn Ala Asn Gly Asp Leu Lys Lys Cys Pro Ile Thr Ile Ser
1               5                   10                  15

Ser Tyr Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Arg Ile
            20                  25                  30

Ala Ala Glu Asn Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr
        35                  40                  45
```

```
Val Asp Ala Leu Ala Ala Gly Leu Thr Asp Glu Asp Met Leu Arg Ile
    50                  55                  60

Leu Asp Glu His Asn Ile Lys Val Thr Glu Val Glu Tyr Ile Thr Gln
65                  70                  75                  80

Trp Gly Thr Ala Glu Asp Arg Thr Ala Glu Gln Gln Lys Lys Glu Gln
                85                  90                  95

Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys His Ile Asn Cys
            100                 105                 110

Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Thr Ala Leu Gly
        115                 120                 125

Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met
    130                 135                 140

Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu
145                 150                 155                 160

Ala Cys Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Ser
                165                 170                 175

Ala Arg Ala Asn Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp
            180                 185                 190

Arg Ile Val Ser Ile Gln Leu Cys Asp Val His Glu Thr Pro Tyr Lys
        195                 200                 205

Glu Leu Arg Glu Glu Ser Leu His Asp Arg Leu Ala Pro Gly Glu Gly
    210                 215                 220

Tyr Gly Asp Thr Val Gly Phe Ala Arg Ile Leu Lys Glu His Gly Val
225                 230                 235                 240

Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp Ser Met Val Glu
                245                 250                 255

Thr Gly Leu Glu Tyr Ala Ala Ile Lys Val Tyr Asn Ala Thr Lys Lys
            260                 265                 270

Val Leu Asp Gln Ala Trp Pro Glu Ile Ser Pro Lys
        275                 280
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 3340 ORF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Met Thr Asn Ala Asn Gly Asn Leu Lys Lys Cys Pro Ile Thr Ile Ser
1               5                   10                  15
```

```
Ser Tyr Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Arg Ile
            20                  25                  30

Ala Ala Glu Asn Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr
        35                  40                  45

Val Asp Ala Leu Ala Ala Gly Leu Thr Asp Glu Asp Met Leu Arg Ile
    50                  55                  60

Leu Asp Glu His His Ile Lys Val Thr Glu Val Glu Tyr Ile Thr Gln
65                  70                  75                  80

Trp Gly Thr Ala Ser Asp Arg Thr Phe Glu Gln Gln Lys Lys Glu Gln
                85                  90                  95

Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys His Ile Asn Cys
            100                 105                 110

Gly Leu Leu Glu Lys Ile Pro Glu Asp Gln Ile Ile Thr Ala Leu Gly
        115                 120                 125

Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met
    130                 135                 140

Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu
145                 150                 155                 160

Ala Cys Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp
                165                 170                 175

Ala Arg Ala Asn Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp
            180                 185                 190

Arg Ile Val Ser Ile Gln Leu Cys Asp Val His Glu Thr Pro Tyr Lys
        195                 200                 205

Glu Leu Arg Glu Glu Ser Leu His Asp Arg Leu Ala Pro Gly Glu Gly
    210                 215                 220

Tyr Gly Asp Thr Ile Gly Phe Ala Arg Ile Leu Lys Glu His Gly Val
225                 230                 235                 240

Ser Pro Arg Val Met Gly Val Glu Val Ile Ser Asp Ser Met Val Glu
                245                 250                 255

Thr Gly Leu Glu Tyr Thr Ala Ile Lys Val Tyr Asn Ala Thr Lys Lys
            260                 265                 270

Val Leu Asp Glu Ala Trp Pro Glu Val Ser Pro Lys
        275                 280
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 3327 ORF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Met Thr Asn Val Asn Gly Asp Leu Lys Lys Cys Pro Ile Thr Ile Ser
1 5 10 15

Ser Tyr Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Arg Ile
20 25 30

Ala Ala Glu Asn Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr
35 40 45

Val Asp Ala Leu Ala Ala Gly Leu Thr Asp Glu Asp Met Leu Arg Ile
50 55 60

Leu Asp Glu His His Ile Lys Val Thr Glu Val Glu Tyr Ile Thr Gln
65 70 75 80

Trp Gly Thr Ala Ser Asp Arg Thr Lys Glu Gln Gln Lys Lys Glu Gln
85 90 95

Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys His Ile Asn Cys
100 105 110

Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Thr Ala Leu Gly
115 120 125

Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met
130 135 140

Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu
145 150 155 160

Ala Cys Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp
165 170 175

Ala Arg Ala Asn Gln Thr Ala Glu Ser Ile Lys Asn Ile Pro Ala Asp
180 185 190

Arg Ile Val Ser Ile Gln Leu Cys Asp Val His Glu Thr Pro Tyr Lys
195 200 205

Glu Leu Arg Glu Glu Ser Leu His Asp Arg Leu Ala Pro Gly Glu Gly
210 215 220

Tyr Gly Asp Thr Val Gly Phe Ala Arg Ile Leu Lys Glu His Gly Val
225 230 235 240

Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp Ser Met Val Glu
245 250 255

Thr Gly Leu Glu Tyr Ala Ala Ile Lys Val Tyr Asn Ala Thr Lys Lys
260 265 270

Val Leu Asp Glu Ala Trp Pro Glu Val Ser Pro Lys
275 280

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: L MONO 3359 ORF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met Thr Asn Ser Asn Gly Asn Leu Lys Lys Cys Pro Ile Thr Ile Ser
1 5 10 15

Ser Tyr Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Lys Ile
20 25 30

Ala Ala Glu Asn Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr
35 40 45

Val Asp Ala Leu Ala Ala Gly Leu Thr Asp Asp Asp Met Leu Gln Ile
50 55 60

Leu Asp Lys His Asn Ile Lys Val Thr Glu Val Glu Tyr Ile Thr Gln
65 70 75 80

Trp Gly Thr Glu Ala Asp Arg Thr Asp Ala Gln Gln Gln Lys Glu Gln
85 90 95

Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys His Ile Asn Cys
100 105 110

Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Thr Ala Leu Gly
115 120 125

Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met
130 135 140

Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu
145 150 155 160

Ala Cys Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp
165 170 175

Ala Arg Ala Asn Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp
180 185 190

Arg Ile Val Ser Ile Gln Leu Cys Asp Val His Glu Thr Pro Tyr Lys
195 200 205

Glu Leu Arg Glu Glu Ser Leu His Asp Arg Leu Pro Pro Gly Glu Gly
210 215 220

Tyr Gly Asp Thr Val Gly Phe Ala Arg Ile Leu Lys Glu His Gly Val
225 230 235 240

Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp Ser Met Val Glu
245 250 255

Thr Gly Leu Glu Tyr Ala Ala Ile Lys Val Tyr Asn Ala Thr Lys Lys
260 265 270

Val Leu Asp Glu Ala Trp Pro Glu Ile Ser Pro Lys
275 280

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GCGATACGAC AAATCTGTTA GGCACC 26

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GAGATACAAC AAAACGATTT GGTACT 26

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GCGAAACCAC GAAACGGTTT GGCACA 26

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GAGATACAAC AAACCGATTA GGTACT 26

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GCGAAACAAC AAAACGGTTA GGTACT 26

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CATTCCTTTC ACAGGGAGTC TTCCTAC 27

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CTTCCCTTTC AAGGTAGAAT CTTCTTGT 28

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CTTTCCTTTC AAAGGGGGAT TTTTA 25

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CTTCCCTTTC AAGATGGAAT CTAGAT 26

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CTTCCCTTTC AAGATAGAAT TTTTTCTT 28

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TAGTTGGATG GAAACAATCC GATCAG 26

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TAGTTGGATA GAAACAATCC GATCAG 26

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

TAATTGGATG GAAACAATCC GGTCAG 26

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

AAGTTGAATA GACACAATTC GGTCAG 26

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CAATTGGATA GAAACAATTC GATCAG 26

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GAGTTGAATT GAAACAATCC GATCAG 26

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TTTGTTGTTC TGCTGTACGA TCTTCGG 27

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TTTGCTGTTC GGCTGTGCGG TCCTCGG 27

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

TTTGTTGTTC TTTGGTGCGG TCGGAAG 27

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GTTGTTGAGC ATCGGTTCGG TCTGCTT 27

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

TTTGTTGCTC GAAAGTGCGG TCAGAAG 27

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

AAATCCCTGA GGAACAAATC ATCGTC 26

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

AAATCCCTGA GGAACAAATC ATTGTC 26

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

AAATCCCCGA AGAACAAATC ATTACG 26

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

AGATTCCGGA AGAACAAATC ATTACT 26

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

AAATTCCAGA AGAGCAAATA ATTACT 26

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

AAATCCCCGA AGACCAAATC ATTACT 26

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GGTAGAATAG GTTAACTGTC CAGTTCC 27

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GGTAGAATAC GTTAACTGTC CAGTTGA 27

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GGTAGAATTC GAAAACTGTC CAGTCAG 27

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GGTAGAATAC GTTAACTGTC CAGTTGT 27

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GGTAGAATGT GAAAACTGTC CAGTCAA 27

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

TACAATTAGA CTGTAGTTAC GCCAGTGA 28

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

TACAATTAAA CTGTAGTTAC GCCAGTGA 28

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TACAATTAGA CTGTAGTTAC ACCAGCAG 28

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

TACAATTAAA CTGTAATTAA GCCAGTGA 28

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

TACAATTAAA CTGTAGTTAC ACCAGCAG 28

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

TACAATTAAA CAGTAGTTAT GCCAGTGA 28

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

TTTGATAGAT TCTGCTGTTT GGTTTGCTCT 30

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TTTGATAGAT TCAGCTGTTT GATTTGCTCT 30

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

TTTGATTGAT TCTGCTGTTT GGTTTGCTCT 30

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TTTGATAGAT TCTGCTGTTT GATTTGCTCT 30

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

TTTGATAGAC TCTGCTGTTT GGTTTGCTCT 30

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

TGCTGTTTGG TTTGCTCTAG CCCAGTGCCA 30

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

AGCTGTTTGA TTTGCTCTAG CCCAGTGCCA 30

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

TGCTGTTTGG TTTGCTCTAG CCGAGTGCCA 30

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

TGCTGTTTGA TTTGCTCTAG CCCAGTGCCA 30

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

TGCTGTTTGG TTTGCTCTTG CCCAGTGCCA 30

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TTGCATTTGT CATAAAAATT ATCTCCTCTC 30

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

TTACATTTGT CATAAAAATT ATCTCCTCTC 30

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

TTGAATTTGT CATAAAAATT ATCTCCTCTC 30

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

CGCTGCGGAA AACGGTTTTG ACGGATTTGG 30

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CGCTGCGGAA AAAGGTTTTG ACGGATTTGG 30

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

CGCAGCAGAA AACGGTTTTG ATGGATTTGG 30

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

TGCAGCAGAA AACGGTTTTG ACGGATTTGG 30

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

AAAAAAATGC CCCATCACGA TTAGCTCTTA 30

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

AAAAAAATGC CCAATCACGA TCAGCTCTTA 30

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

AAAAAAATGC CCCATCACGA TTAGTTCTTA 30

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

AAATGCAAAT GGCAACCTAA AAAAATGCCC 30

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

AAATGCAAAT GGCAATCTAA AAAAATGCCC 30

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

AAATGCAAAT GGCGACCTAA AAAAATGCCC 30

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

AAATGTAAAT GGCGACTTAA AAAAATGCCC 30

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

AAATTCAAAT GGCAACTTAA AAAAATGCCC 30

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

CAATACCAAT TTGTTTTTAT GGAATAGTCA 30

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

TAATACCAAT TTGTTTTTAT GGAATAGTCA 30

## CLAIMS

What is claimed is:

1. A method for determining whether an unknown bacterium is a *Listeria monocytogenes*, comprising

(A) amplifying genomic DNA from (i) a positive test panel of *Listeria monocytogenes* strains and (ii) a negative test panel of non- *monocytogenes Listeria* strains with a primer derived from a pre-marker diagnostic fragment for *Listeria monocytogenes* selected from the group of nucleic acids corresponding to SEQ ID NOS:17, 18, and 19 to yield a 1300 bp diagnostic fragment for each of the positive and negative test panels;

(B) selecting at least one *Listeria monocytogenes* diagnostic marker contained within the diagnostic fragment by comparing the diagnostic fragment obtained from the amplification of the positive test panel with the diagnostic fragment obtained from the amplification of the negative test panel whereby at least one highly conserved region in the diagnostic fragment of the positive test panel is identified which is less than 90% homologous to any member of the negative test panel;

(C) designing at least one amplification primer corresponding to the at least one diagnostic marker identified in step (B); and

(D) amplifying genomic DNA of the unknown bacterium under suitable annealing temperatures with the at least one amplification primer of step (C), whereby obtaining at least one amplification product indicates that the unknown bacterium is a *Listeria monocytogenes*.

2. The method of Claim 1 wherein the *Listeria monocytogenes* pre-marker diagnostic fragment is selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:20-23.

3. The method of Claim 1 wherein the diagnostic fragment is at least 83% homologous to SEQ ID NOS:24-31 and 33-40.

4. The method of Claim 1 wherein the diagnostic fragment is selected from the group of consisting nucleic acids corresponding to SEQ ID NOS:24-31 and 33-40.

5. The method of Claim 1 wherein at least one diagnostic marker selected in step (B) is selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:46-83.

6. The method of Claim 1 wherein the at least one amplification primer is about 15 to 30 bp in length.

7. The method of Claim 1 wherein the suitable annealing temperature is in the range of about 60 °C to 70 °C.

8. A method for determining whether an unknown bacterium is a member of the genus *Listeria*, comprising

(A) amplifying genomic DNA from (i) a positive test panel of *Listeria monocytogenes* strains and (ii) a negative test panel of non-*monocytogenes Listeria* strains with a primer derived from a pre-marker diagnostic fragment for *Listeria monocytogenes* strains selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:17, 18, and 19 to yield a 1300 bp diagnostic fragment for each of the positive and negative test panels;

(B) selecting at least one *Listeria* genus-specific diagnostic marker contained within the diagnostic fragment by comparing the diagnostic fragment obtained from the amplification of the positive test panel with the diagnostic fragment obtained from the amplification of the negative test panel whereby at least one highly conserved region in the diagnostic fragment of the positive test panel is identified which is at least 90% homologous to the corresponding positive test panel of diagnostic fragment;

(C) designing amplification primers corresponding to the at least one *Listeria* genus-specific diagnostic marker selected in step (B); and

(D) amplifying genomic DNA of the unknown bacterium under suitable annealing temperatures with the amplification primers of step (C), whereby obtaining amplification products indicates that the unknown bacterium is a member of the genus *Listeria*.

9. The method of Claim 8 wherein at step (A) the diagnostic fragment is at least 83% homologous to any one of SEQ ID NOS:24-31 and 33-40.

10. The method of Claim 8 wherein at step (A) the diagnostic fragment is selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:24-31 and 33-40.

11. The method of Claim 8 wherein the *Listeria monocytogenes* pre-marker diagnostic fragment is selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:20-23.

12. The method of Claim 8 wherein the at least one diagnostic marker selected in step (B) is selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:84-110.

13. The method of Claim 8 wherein the at least one amplification primer is about 15 to 30 bp in length.

14. The method of Claim 8 wherein the suitable annealing temperature is in the range of about 60 °C to 70 °C.

15. A method for determining whether an unknown bacterium is a *Listeria monocytogenes,* comprising contacting the genomic DNA of the unknown bacterium with a nucleic acid probe selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:46-83, and then detecting hybridization of the nucleic acid probe with the genomic DNA.

16. A method for determining whether an unknown bacterium is a *Listeria monocytogenes* comprising contacting the genomic DNA of the unknown bacterium with a nucleic acid probe selected from the group consisting of nucleic acids corresponding to SEQ ID NOS:84-110, and then detecting hybridization of the nucleic acid probe with the genomic DNA.

17. Isolated nucleic acid fragments selected from the group consisting of nucleic acid fragments corresponding to SEQ ID NOS:17 through 110.

18. An isolated nucleic acid fragment encoding the amino acid sequence as given in any one of SEQ ID NOS:32 and 41-45.

19. A nucleic acid fragment located on a diagnostic fragment of about 1300 bp and selected from the group consisting of nucleic acid fragments designated

1515(rc341x2)-26-363,
1515(rc341x2)-27-281,
1515-26-36,
1515-27-357,
1515-26-rc233,
1515(8585)-27-rc737,
1515(8585)-28-rc793
1515-30-76,

1515-30-88,
1515(8585)-30-624,
1515(8585)-30-rc483,
1515(8585)-30-rc555,
1515(8585)-30-rc573,
1515(8585)-30-rc824,

the diagnostic fragment characterized by

(A) at least 83% homology to any one of SEQ ID NOS:24-31 and 33-40; and

(B) an open reading frame of about 855 bp contained within the diagnostic fragment, the open reading frame encoding an amino acid sequence of any one of SEQ ID NOS:32 and 41-45.

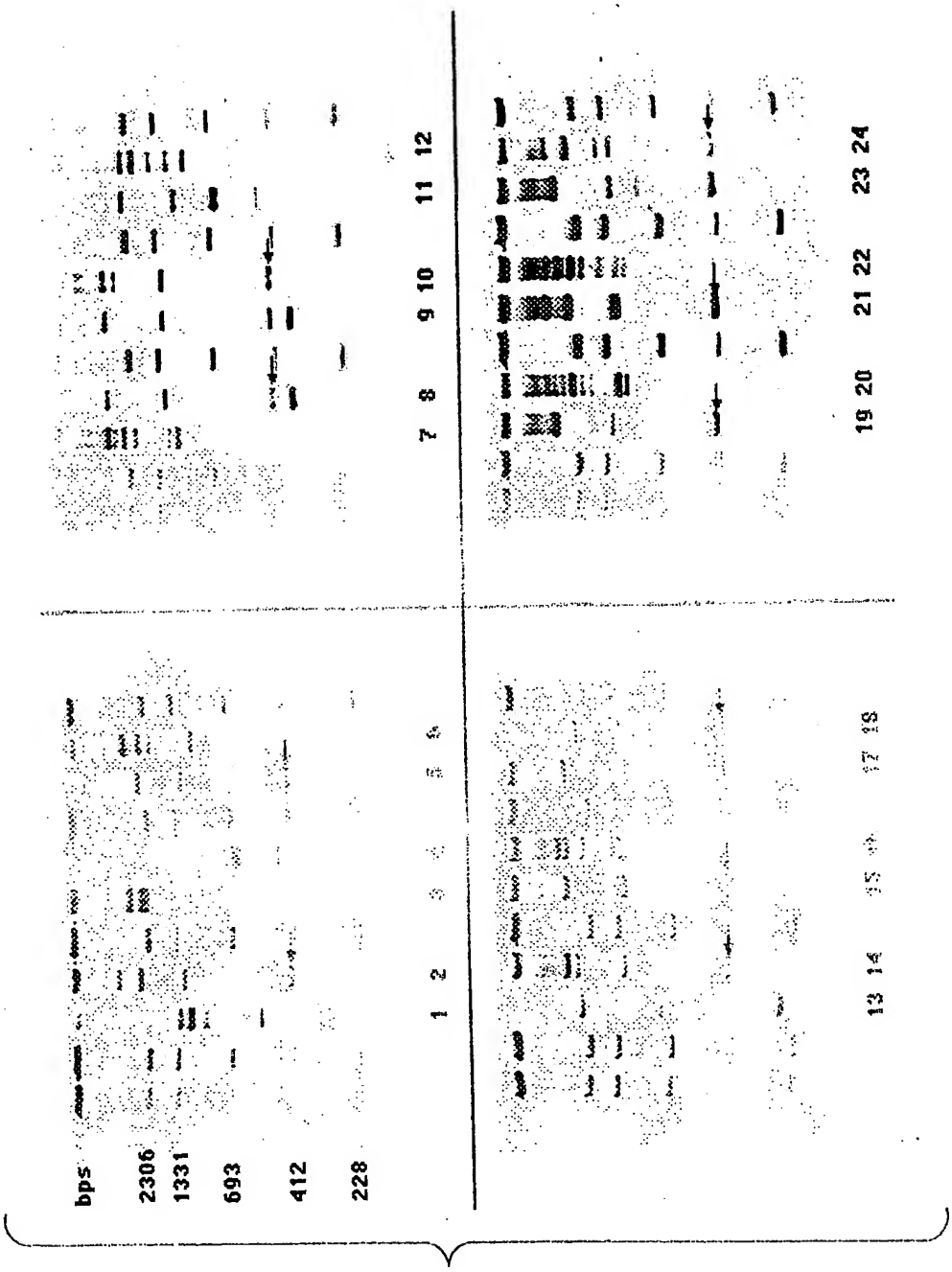


FIG. 1

| | |
|---|---|
| L. monocytogenes #647 | Met Thr Asn Ala Asn Gly Asn Leu Lys Lys Cys Pro Ile Thr Ile Ser Ser Tyr |
| L. innocua #4450 | Met Thr Asn Ala Asn Gly Asp Leu Lys Lys Cys Pro Ile Thr Ile Ser Ser Tyr |
| L. ivanovii #3340 | Met Thr Asn Ala Asn Gly Asn Leu Lys Lys Cys Pro Ile Thr Ile Ser Ser Tyr |
| L. seeligeri #3327 | Met Thr Asn Val Asn Gly Asp Leu Lys Lys Cys Pro Ile Thr Ile Ser Ser Tyr |
| L. welshimeri #3359 | Met Thr Asn Ser Asn Gly Asn Leu Lys Lys Cys Pro Ile Thr Ile Ser Ser Tyr |
| | |
| L. monocytogenes #647 | Thr Leu Gly Thr Glu Val Ser Phe Pro Lys Arg Val Lys Val Ala Ala Glu Asn |
| L. innocua #4450 | Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Arg Ile Ala Ala Glu Asn |
| L. ivanovii #3340 | Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Arg Ile Ala Ala Glu Asn |
| L. seeligeri #3327 | Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Arg Ile Ala Ala Glu Asn |
| L. welshimeri #3359 | Thr Leu Gly Thr Glu Val Ser Phe Pro Glu Arg Val Lys Ile Ala Ala Glu Asn |
| | |
| L. monocytogenes #647 | Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr Val Asp Ala Leu Ala Ala |
| L. innocua #4450 | Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr Val Asp Ala Leu Ala Ala |
| L. ivanovii #3340 | Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr Val Asp Ala Leu Ala Ala |
| L. seeligeri #3327 | Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr Val Asp Ala Leu Ala Ala |
| L. welshimeri #3359 | Gly Phe Asp Gly Ile Gly Leu Arg Ala Glu Asn Tyr Val Asp Ala Leu Ala Ala |
| | |
| L. monocytogenes #647 | Gly Leu Thr Asp Glu Asp Met Leu Arg Ile Leu Asp Glu His Asn Met Lys Val |
| L. innocua #4450 | Gly Leu Thr Asp Glu Asp Met Leu Arg Ile Leu Asp Glu His Asn Ile Lys Val |
| L. ivanovii #3340 | Gly Leu Thr Asp Glu Asp Met Leu Arg Ile Leu Asp Glu His His Ile Lys Val |
| L. seeligeri #3327 | Gly Leu Thr Asp Glu Asp Met Leu Arg Ile Leu Asp Glu His His Ile Lys Val |
| L. welshimeri #3359 | Gly Leu Thr Asp Asp Asp Met Leu Gln Ile Leu Asp Lys His Asn Ile Lys Val |
| | |
| L. monocytogenes #647 | Thr Glu Val Glu Tyr Ile Thr Gln Trp Gly Thr Ala Glu Asp Arg Thr Ala Glu |
| L. innocua #4450 | Thr Glu Val Glu Tyr Ile Thr Gln Trp Gly Thr Ala Glu Asp Arg Thr Ala Glu |
| L. ivanovii #3340 | Thr Glu Val Glu Tyr Ile Thr Gln Trp Gly Thr Ala Ser Asp Arg Thr Phe Glu |
| L. seeligeri #3327 | Thr Glu Val Glu Tyr Ile Thr Gln Trp Gly Thr Ala Ser Asp Arg Thr Lys Glu |
| L. welshimeri #3359 | Thr Glu Val Glu Tyr Ile Thr Gln Trp Gly Thr Glu Ala Asp Arg Thr Asp Ala |

FIG. 2

FIG.2
(CONTINUED)

| | |
|---|---|
| L. monocytogenes #647 | Gln Gln Lys Lys Glu Gln Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys |
| L. innocua #4450 | Gln Gln Lys Lys Glu Gln Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys |
| L. ivanovii #3340 | Gln Gln Lys Lys Glu Gln Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys |
| L. seeligeri #3327 | Gln Gln Lys Lys Glu Gln Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys |
| L. welshimeri #3359 | Gln Gln Gln Lys Glu Gln Thr Thr Phe His Met Ala Arg Leu Phe Gly Val Lys |
| L. monocytogenes #647 | His Ile Asn Cys Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Val Ala |
| L. innocua #4450 | His Ile Asn Cys Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Thr Ala |
| L. ivanovii #3340 | His Ile Asn Cys Gly Leu Leu Glu Lys Ile Pro Glu Asp Gln Ile Ile Thr Ala |
| L. seeligeri #3327 | His Ile Asn Cys Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Thr Ala |
| L. welshimeri #3359 | His Ile Asn Cys Gly Leu Leu Glu Lys Ile Pro Glu Glu Gln Ile Ile Thr Ala |
| L. monocytogenes #647 | Leu Gly Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met |
| L. innocua #4450 | Leu Gly Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met |
| L. ivanovii #3340 | Leu Gly Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met |
| L. seeligeri #3327 | Leu Gly Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met |
| L. welshimeri #3359 | Leu Gly Glu Leu Cys Asp Arg Ala Glu Glu Leu Ile Ile Gly Leu Glu Phe Met |
| L. monocytogenes #647 | Pro Tyr Ser Gly Val Ala Asp Leu Gln Ala Ala Trp Arg Val Ala Glu Ala Cys |
| L. innocua #4450 | Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu Ala Cys |
| L. ivanovii #3340 | Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu Ala Cys |
| L. seeligeri #3327 | Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu Ala Cys |
| L. welshimeri #3359 | Pro Tyr Ser Gly Val Ala Asp Leu Ala Ala Ala Trp Arg Val Ala Glu Ala Cys |
| L. monocytogenes #647 | Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp Ala Arg Ala Asn |
| L. innocua #4450 | Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Ser Ala Arg Ala Asn |
| L. ivanovii #3340 | Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp Ala Arg Ala Asn |
| L. seeligeri #3327 | Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp Ala Arg Ala Asn |
| L. welshimeri #3359 | Gly Arg Asp Asn Ala Gln Leu Ile Cys Asp Thr Trp His Trp Ala Arg Ala Asn |

FIG.2
(CONTINUED)

| | |
|---|---|
| L. monocytogenes #647 | Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp Arg Ile Val Ser Ile Gln |
| L. innocua #4450 | Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp Arg Ile Val Ser Ile Gln |
| L. ivanovii #3340 | Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp Arg Ile Val Ser Ile Gln |
| L. seeligeri #3327 | Gln Thr Ala Glu Ser Ile Lys Asn Ile Pro Ala Asp Arg Ile Val Ser Ile Gln |
| L. welshimeri #3359 | Gln Thr Ala Glu Ser Ile Lys Asn Val Pro Ala Asp Arg Ile Val Ser Ile Gln |
| L. monocytogenes #647 | Leu Cys Asp Val His Glu Thr Pro Tyr Lys Glu Leu Arg Glu Glu Ser Leu His |
| L. innocua #4450 | Leu Cys Asp Val His Glu Thr Pro Tyr Lys Glu Leu Arg Glu Glu Ser Leu His |
| L. ivanovii #3340 | Leu Cys Asp Val His Glu Thr Pro Tyr Lys Glu Leu Arg Glu Glu Ser Leu His |
| L. seeligeri #3327 | Leu Cys Asp Val His Glu Thr Pro Tyr Lys Glu Leu Arg Glu Glu Ser Leu His |
| L. welshimeri #3359 | Leu Cys Asp Val His Glu Thr Pro Tyr Lys Glu Leu Arg Glu Glu Ser Leu His |
| L. monocytogenes #647 | Asp Arg Leu Ala Pro Gly Glu Gly Tyr Gly Asp Thr Val Gly Phe Ala Lys Ile |
| L. innocua #4450 | Asp Arg Leu Ala Pro Gly Glu Gly Tyr Gly Asp Thr Val Gly Phe Ala Arg Ile |
| L. ivanovii #3340 | Asp Arg Leu Ala Pro Gly Glu Gly Tyr Gly Asp Thr Ile Gly Phe Ala Arg Ile |
| L. seeligeri #3327 | Asp Arg Leu Ala Pro Gly Glu Gly Tyr Gly Asp Thr Val Gly Phe Ala Arg Ile |
| L. welshimeri #3359 | Asp Arg Leu Pro Pro Gly Glu Gly Tyr Gly Asp Thr Val Gly Phe Ala Arg Ile |
| L. monocytogenes #647 | Leu Lys Glu His Gly Val Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp |
| L. innocua #4450 | Leu Lys Glu His Gly Val Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp |
| L. ivanovii #3340 | Leu Lys Glu His Gly Val Ser Pro Arg Val Met Gly Val Glu Val Ile Ser Asp |
| L. seeligeri #3327 | Leu Lys Glu His Gly Val Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp |
| L. welshimeri #3359 | Leu Lys Glu His Gly Val Asn Pro Arg Val Met Gly Val Glu Val Ile Ser Asp |
| L. monocytogenes #647 | Ser Met Val Ala Thr Gly Leu Glu Tyr Ala Ala Leu Lys Val Tyr Asn Ala Thr |
| L. innocua #4450 | Ser Met Val Glu Thr Gly Leu Glu Tyr Ala Ala Ile Lys Val Tyr Asn Ala Thr |
| L. ivanovii #3340 | Ser Met Val Glu Thr Gly Leu Glu Tyr Thr Ala Ile Lys Val Tyr Asn Ala Thr |
| L. seeligeri #3327 | Ser Met Val Glu Thr Gly Leu Glu Tyr Ala Ala Ile Lys Val Tyr Asn Ala Thr |
| L. welshimeri #3359 | Ser Met Val Glu Thr Gly Leu Glu Tyr Ala Ala Ile Lys Val Tyr Asn Ala Thr |
| L. monocytogenes #647 | Lys Lys Val Leu Asp Glu Ala Trp Pro Glu Ile Ser Pro Arg TER (SEQ ID NO: 41) |
| L. innocua #4450 | Lys Lys Val Leu Asp Gln Ala Trp Pro Glu Ile Ser Pro Lys TER (SEQ ID NO: 42) |
| L. ivanovii #3340 | Lys Lys Val Leu Asp Glu Ala Trp Pro Glu Val Ser Pro Lys TER (SEQ ID NO: 43) |
| L. seeligeri #3327 | Lys Lys Val Leu Asp Glu Ala Trp Pro Glu Val Ser Pro Lys TER (SEQ ID NO: 44) |
| L. welshimeri #3359 | Lys Lys Val Leu Asp Glu Ala Trp Pro Glu Ile Ser Pro Lys TER (SEQ ID NO: 45) |

FIG.3

| | | |
|---|---|---|
| 1515(rc341x2)-26-363 | | |
| L. monocytogenes #647 | GCG ATA CGA CAA ATC TGT TAG GCA CC | (SEQ ID NO: 46) |
| L. innocua #4450 | GCG ATA CGA CAA ATC TGT TAG GCA CC | (SEQ ID NO: 46) |
| L. seeligeri #3327 | GAG ATA CAA CAA AAC GAT TIG GIA CI | (SEQ ID NO: 47) |
| L. welsh. #3359 | GCG AAA CCA CGA AAC GGT TIG GCA CA | (SEQ ID NO: 48) |
| L. ivanovii #3340 | GAG ATA CAA CAA ACC GAT TAG GIA CI | (SEQ ID NO: 49) |
| | GCG AAA CAA CAA AAC GGT TAG GIA CI | (SEQ ID NO: 50) |
| 1515(rc341x2)-27-281 | | |
| L. monocytogenes #647 | CAT TCC TTT CAC AGG GAG TCT TCC TAC | (SEQ ID NO: 51) |
| L. monocytogenes #1324 | CAT TCC TTT CAC AGG GAG TCT TCC TAC | (SEQ ID NO: 51) |
| L. innocua #4450 | CAT TCC TTT CAC AGG GAG TCT TCC TAC | (SEQ ID NO: 51) |
| L. seeligeri #3327 | C-T TCC CTTT CA- AGGIA GAA TCT TCI TGI | (SEQ ID NO: 52) |
| L. welsh. #3359 | C-T TIC CTTT CAA AGGGG GAI TIT T-- -A- | (SEQ ID NO: 53) |
| L. ivanovii #3340 | C-T TCC CTTT CA- AGAIG GAA TCT AG- -AI | (SEQ ID NO: 54) |
| | C-T TCC CTTT CA- AGAIA GAA TIT TII CII | (SEQ ID NO: 55) |
| 1515-26-36 | | |
| L. monocytogenes #647 | TAG TTG GAT GGA AAC AAT CCG ATC AG | (SEQ ID NO: 56) |
| L. monocytogenes #1324 | TAG TTG GAT GGA AAC AAT CCG ATC AG | (SEQ ID NO: 56) |
| L. innocua #4450 | TAG TTG GAT AGA AAC AAT CCG ATC AG | (SEQ ID NO: 57) |
| L. seeligeri #3327 | TAA TTG GAT GGA AAC AAT CCG GTC AG | (SEQ ID NO: 58) |
| L. welsh. #3359 | AAG TTG AAT AGA CAC AAT ICG GTC AG | (SEQ ID NO: 59) |
| L. ivanovii #3340 | CAA TTG GAT AGA AAC AAT ICG ATC AG | (SEQ ID NO: 60) |
| | GAG TTG AAT IGA AAC AAT CCG ATC AG | (SEQ ID NO: 61) |
| 1515-27-357 | | |
| L. monocytogenes #647 | TTT GTT GTT CTG CTG TAC GAT CTT CGG | (SEQ ID NO: 62) |
| L. monocytogenes #1324 | TTT GTT GTT CTG CTG TAC GAT CTT CGG | (SEQ ID NO: 62) |
| L. innocua #4450 | TTT GTT GTT CTG CTG TAC GAT CTT CGG | (SEQ ID NO: 62) |
| L. seeligeri #3327 | TTT GCT GTT CGG CTG TGC GGT CCT CGG | (SEQ ID NO: 63) |
| L. welsh. #3359 | TTT GTT GTT CTI IGG TGC GGT CGG AAG | (SEQ ID NO: 64) |
| L. ivanovii #3340 | GTT GTT GAG CAI CGG TIC GGT CTG CII | (SEQ ID NO: 65) |
| | TTT GTT GCT CGA AAG TGC GGT CAG AAG | (SEQ ID NO: 66) |

| | | |
|---|---|---|
| 1515-26-rc233 | AAA TCC CTG AGG AAC AAA TCA TCG TC | (SEQ ID NO: 67) |
| L. monocytogenes #647 | AAA TCC CTG AGG AAC AAA TCA TCG TC | (SEQ ID NO: 67) |
| L. monocytogenes #1324 | AAA TCC CTG AGG AAC AAA TCA ITG TC | (SEQ ID NO: 68) |
| L. innocua #4450 | AAA TCC CCG AAG AAC AAA TCA TIA CG | (SEQ ID NO: 69) |
| L. seeligeri #3327 | AGA TIC CGG AAG AAC AAA TCA TIA CI | (SEQ ID NO: 70) |
| L. welsh. #3359 | AAA TIC CAG AAG AGC AAA TAA TIA CI | (SEQ ID NO: 71) |
| L. ivanovii #3340 | AAA TCC CCG AAG ACC AAA TCA TIA CI | (SEQ ID NO: 72) |
| 1515(8585)-27-rc737 | GGT AGA ATA GGT TAA CTG TCC AGT TCC | (SEQ ID NO: 73) |
| L. monocytogenes #647 | GGT AGA ATA GGT TAA CTG TCC AGT TCC | (SEQ ID NO: 73) |
| L. monocytogenes #1324 | GGT AGA ATA GGT TAA CTG TCC AGT TCC | (SEQ ID NO: 73) |
| L. innocua #4450 | GGT AGA ATA CGT TAA CTG TCC AGT TGA | (SEQ ID NO: 74) |
| L. seeligeri #3327 | GGT AGA ATI CGA AAA CTG TCC AGT CAG | (SEQ ID NO: 75) |
| L. welsh. #3359 | GGT AGA ATA CGT TAA CTG TCC AGT TGI | (SEQ ID NO: 76) |
| L. ivanovii #3340 | GGT AGA ATG IGA AAA CTG TCC AGT CAA | (SEQ ID NO: 77) |
| 1515(8585)-28-rc793 | TAC AAT TAG ACT GTA GTT ACG CCA GTG A | (SEQ ID NO: 78) |
| L. monocytogenes #647 | TAC AAT TAG ACT GTA GTT ACG CCA GTG A | (SEQ ID NO: 78) |
| L. monocytogenes #1324 | TAC AAT TAA ACT GTA GTT ACG CCA GTG A | (SEQ ID NO: 79) |
| L. innocua #4450 | TAC AAT TAG ACT GTA GTT ACA CCA GCA G | (SEQ ID NO: 80) |
| L. seeligeri #3327 | TAC AAT TAA ACT GTA ATT AAG CCA GTG A | (SEQ ID NO: 81) |
| L. welsh. #3359 | TAC AAT TAA ACT GTA GTT ACA CCA GCA G | (SEQ ID NO: 82) |
| L. ivanovii #3340 | TAC AAT TAA ACA GTA GTT AIG CCA GTG A | (SEQ ID NO: 83) |

FIG. 3
(CONTINUED)

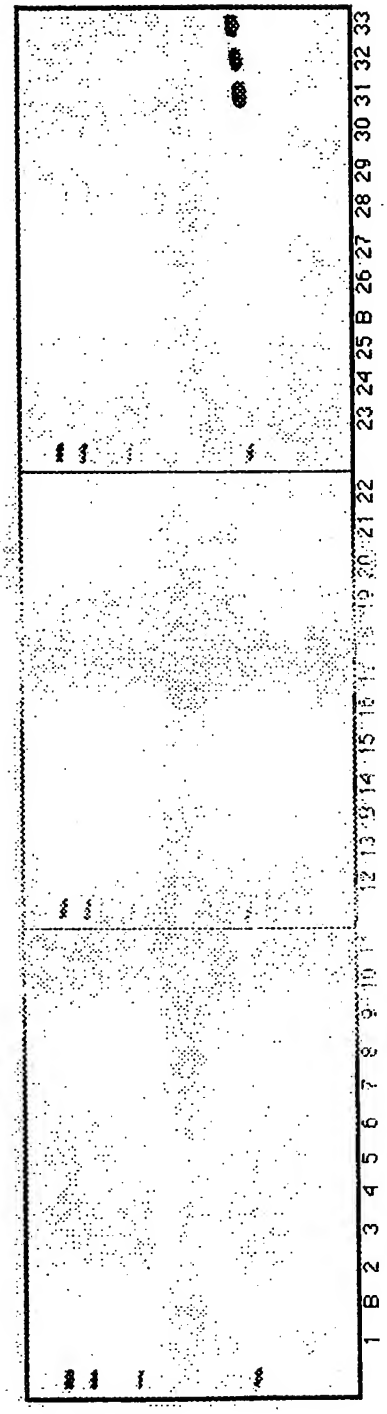
1 B 2 3 4 5 6 7 8 9 10 11 12 13 B 14 15 16 17 18 19 20 21 22 23 24 25 B 26 27 28 29 30 31 32 33

FIG. 4A

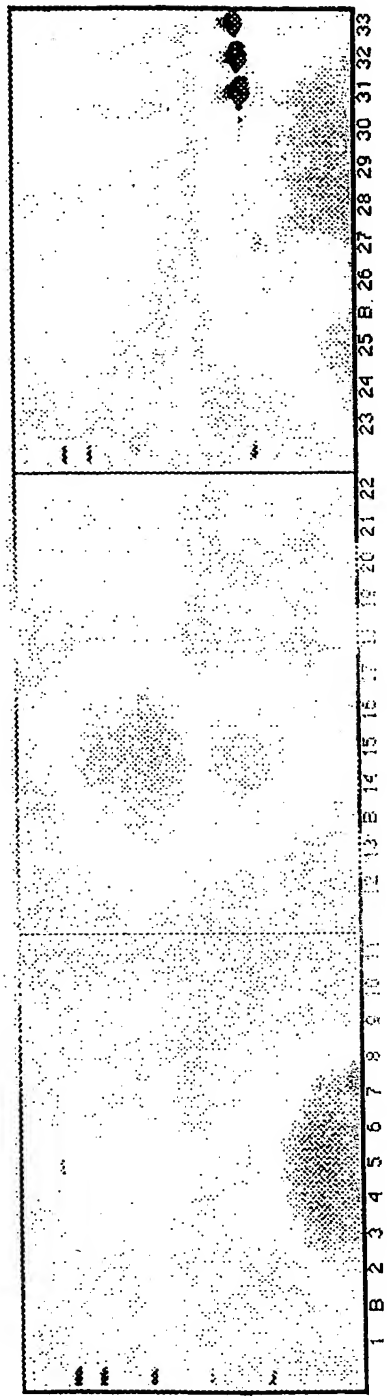
1 B 2 3 4 5 6 7 8 9 10 11 12 13 B 14 15 16 17 18 19 20 21 22 23 24 25 B 26 27 28 29 30 31 32 33

FIG. 4B

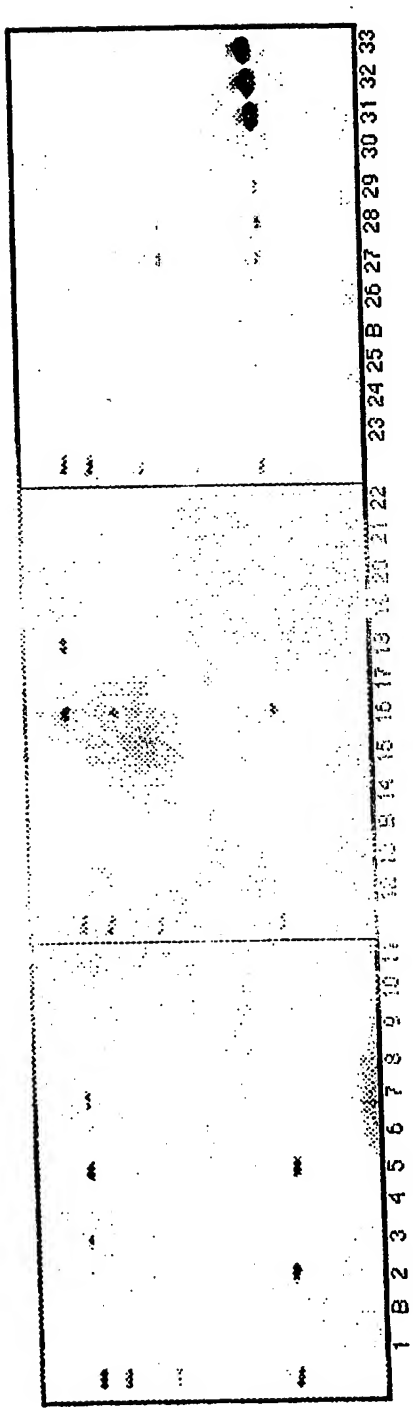


FIG. 4C

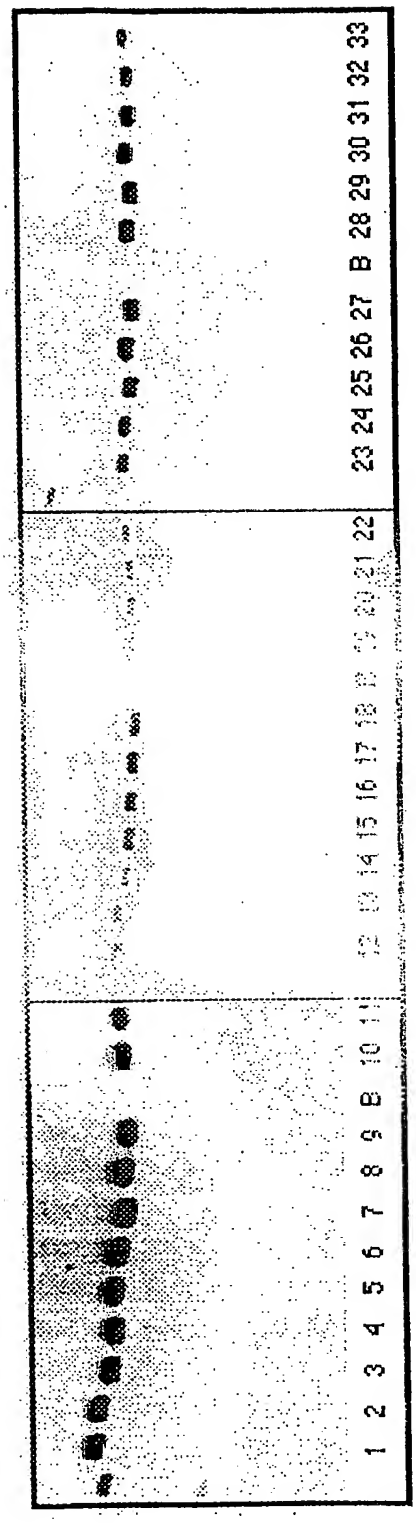
1 2 3 4 5 6 7 8 9 B 10 11
12 13 14 15 16 17 18 19 20 21 22
23 24 25 26 27 B 28 29 30 31 32 33

FIG. 5A

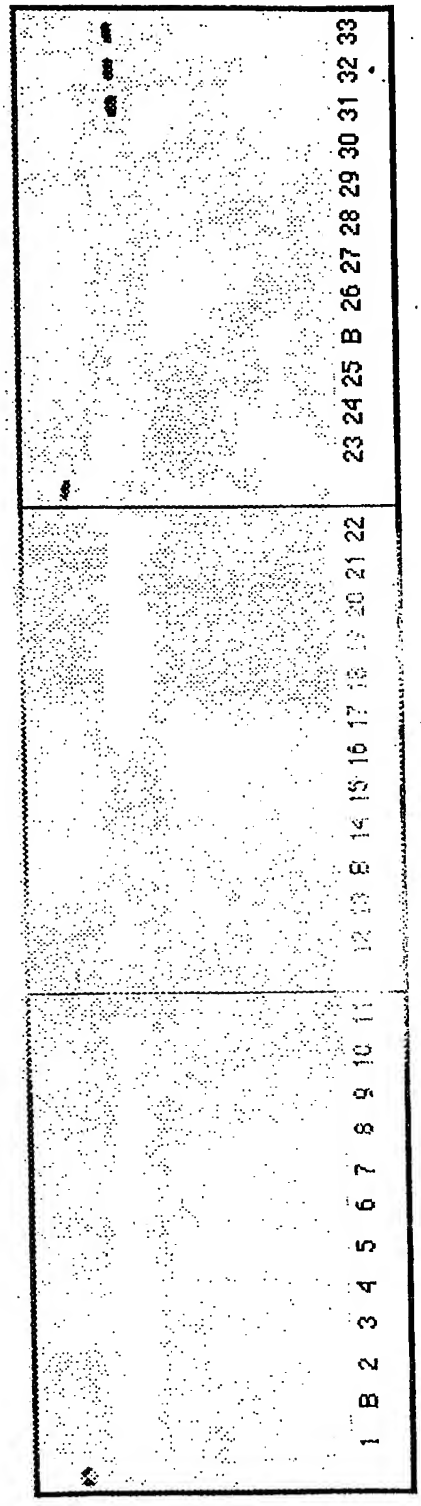
1 B 2 3 4 5 6 7 8 9 10 11
12 13 B 14 15 16 17 18 19 20 21 22
23 24 25 B 26 27 28 29 30 31 32 33

FIG. 5B

FIG.6

| | | |
|---|---|---|
| 1515-30-76 | | |
| L. monocytogenes #647. | TTT GAT AGA TTC TGC TGT TTG GTT TGC TCT | (SEQ ID NO: 84) |
| L. monocytogenes #1324. | TTT GAT AGA TTC TGC TGT TTG GTT TGC TCT | (SEQ ID NO: 84) |
| L. innocua #4450 | TTT GAT AGA TTC AGC TGT TTG ATT TGC TCT | (SEQ ID NO: 85) |
| L. seeligeri #3327 | TTT GAT IGA TTC TGC TGT TTG GTT TGC TCT | (SEQ ID NO: 86) |
| L. ivanovii #3340 | TTT GAT AGA TTC TGC TGT TTG ATT TGC TCT | (SEQ ID NO: 87) |
| L. welshimeri #3359 | TTT GAT AGA TTC TGC TGT TTG GTT TGC TCT | (SEQ ID NO: 84) |
| | TTT GAT AGA CTC TGC TGT TTG GTT TGC TCT | (SEQ ID NO: 88) |
| 1515-30-88 | | |
| L. monocytogenes #647. | TGC TGT TTG GTT TGC TCT AGC CCA GTG CCA | (SEQ ID NO: 89) |
| L. monocytogenes #1324. | TGC TGT TTG GTT TGC TCT AGC CCA GTG CCA | (SEQ ID NO: 89) |
| L. innocua #4450 | AGC TGT TTG ATT TGC TCT AGC CCA GTG CCA | (SEQ ID NO: 90) |
| L. seeligeri #3327 | TGC TGT TTG GTT TGC TCT AGC CGA GTG CCA | (SEQ ID NO: 91) |
| L. ivanovii #3340 | TGC TGT TTG ATT TGC TCT AGC CCA GTG CCA | (SEQ ID NO: 92) |
| L. welshimeri #3359 | TGC TGT TTG GTT TGC TCT IGC CCA GTG CCA | (SEQ ID NO: 93) |
| | TGC TGT TTG GTT TGC TCT IGC CCA GTG CCA | (SEQ ID NO: 93) |
| 1515(8585)-30-624 | | |
| L. monocytogenes #647. | TTG CAT TTG TCA TAA AAA TTA TCT CCT CTC | (SEQ ID NO: 94) |
| L. monocytogenes #1324 | TTG CAT TTG TCA TAA AAA TTA TCT CCT CTC | (SEQ ID NO: 94) |
| L. innocua #4450 | TTG CAT TTG TCA TAA AAA TTA TCT CCT CTC | (SEQ ID NO: 94) |
| L. seeligeri #3327 | TTG CAT TTG TCA TAA AAA TTA TCT CCT CTC | (SEQ ID NO: 94) |
| L. ivanovii #3340 | TTA CAT TTG TCA TAA AAA TTA TCT CCT CTC | (SEQ ID NO: 95) |
| L. welshimeri #3359 | TTG CAT TTG TCA TAA AAA TTA TCT CCT CTC | (SEQ ID NO: 94) |
| | TTG AAT TTG TCA TAA AAA TTA TCT CCT CTC | (SEQ ID NO: 96) |
| 1515(8585)-30-rc483 | | |
| L. monocytogenes #647 | CGC TGC GGA AAA CGG TTT TGA CGG ATT TGG | (SEQ ID NO: 97) |
| L. monocytogenes #1324. | CGC TGC GGA AAA CGG TTT TGA CGG ATT TGG | (SEQ ID NO: 97) |
| L. innocua #4450 | CGC TGC GGA AAA AGG TTT TGA CGG ATT TGG | (SEQ ID NO: 98) |
| L. seeligeri #3327 | CGC AGC AGA AAA CGG TTT TGA IGG ATT TGG | (SEQ ID NO: 99) |
| L. ivanovii #3340 | CGC AGC AGA AAA CGG TTT TGA IGG ATT TGG | (SEQ ID NO: 99) |
| L. welshimeri #3359 | IGC AGC AGA AAA CGG TTT TGA CGG ATT TGG | (SEQ ID NO: 100) |
| | IGC AGC AGA AAA CGG TTT TGA CGG ATT TGG | (SEQ ID NO: 100) |

| | | |
|---|---|---|
| 1515(8585)-30-rc555 | AAA AAA ATG CCC CAT CAC GAT TAG CTC TTA | (SEQ ID NO: 101) |
| L. monocytogenes #647 | AAA AAA ATG CCC CAT CAC GAT TAG CTC TTA | (SEQ ID NO: 101) |
| L. monocytogenes #1324. | AAA AAA ATG CCC CAT CAC GAT TAG CTC TTA | (SEQ ID NO: 101) |
| L. innocua #4450 | AAA AAA ATG CCC AAT CAC GAT CAG CTC TTA | (SEQ ID NO: 102) |
| L. seeligeri #3327 | AAA AAA ATG CCC CAT CAC GAT TAG ITC TTA | (SEQ ID NO: 103) |
| L. ivanovii #3340 | AAA AAA ATG CCC CAT CAC GAT TAG CTC TTA | (SEQ ID NO: 101) |
| L. welshimeri #3359 | AAA AAA ATG CCC CAT CAC GAT TAG ITC TTA | (SEQ ID NO: 103) |
| 1515(8585)-30-rc573 | AAA TGC AAA TGG CAA CCT AAA AAA ATG CCC | (SEQ ID NO: 104) |
| L. monocytogenes #647 | AAA TGC AAA TGG CAA CCT AAA AAA ATG CCC | (SEQ ID NO: 104) |
| L. monocytogenes #1324. | AAA TGC AAA TGG CAA ICT AAA AAA ATG CCC | (SEQ ID NO: 105) |
| L. innocua #4450 | AAA TGC AAA TGG CGA CCT AAA AAA ATG CCC | (SEQ ID NO: 106) |
| L. seeligeri #3327 | AAA TGI AAA TGG CGA CIT AAA AAA ATG CCC | (SEQ ID NO: 107) |
| L. ivanovii #3340 | AAA TGC AAA TGG CAA CCT AAA AAA ATG CCC | (SEQ ID NO: 104) |
| L. welshimeri #3359 | AAA TIC AAA TGG CAA CIT AAA AAA ATG CCC | (SEQ ID NO: 108) |
| 1515(8585)-30-rc824 | CAA TAC CAA TTT GTT TTT ATG GAA TAG TCA | (SEQ ID NO: 109) |
| L. monocytogenes #647. | CAA TAC CAA TTT GTT TTT ATG GAA TAG TCA | (SEQ ID NO: 109) |
| L. monocytogenes #1324 | CAA TAC CAA TTT GTT TTT ATG GAA TAG TCA | (SEQ ID NO: 109) |
| L. innocua #4450 | IAA TAC CAA TTT GTT TTT ATG GAA TAG TCA | (SEQ ID NO: 110) |
| L. seeligeri #3327 | IAA TAC CAA TTT GTT TTT ATG GAA TAG TCA | (SEQ ID NO: 110) |
| L. ivanovii #3340 | IAA TAC CAA TTT GTT TTT ATG GAA TAG TCA | (SEQ ID NO: 110) |
| L. welshimeri #3359 | IAA TAC CAA TTT GTT TTT ATG GAA TAG TCA | (SEQ ID NO: 110) |

FIG. 6
(CONTINUED)

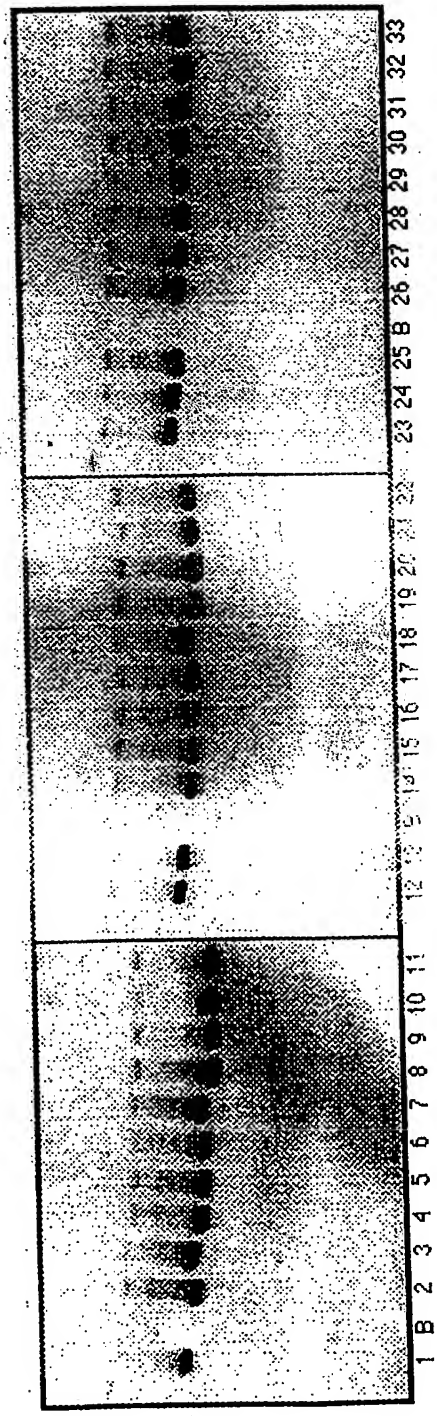
1 B 2 3 4 5 6 7 8 9 10 11
12 13 14 15 16 17 18 19 20 21 22
23 24 25 B 26 27 28 29 30 31 32 33

FIG. 7

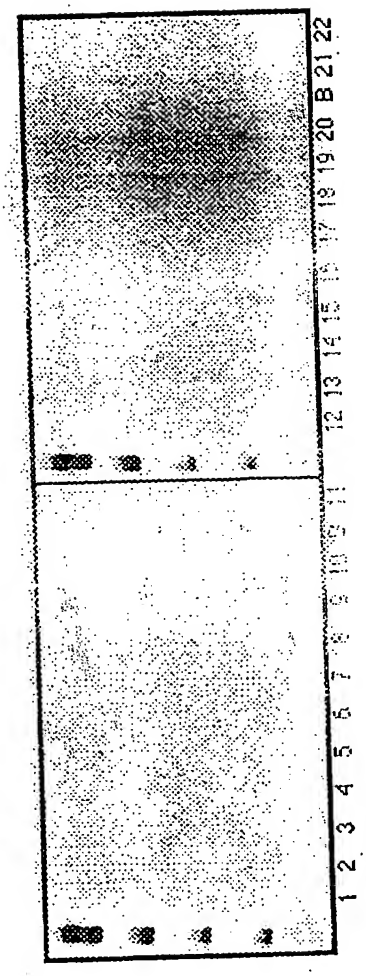
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 B 21 22

FIG. 8A

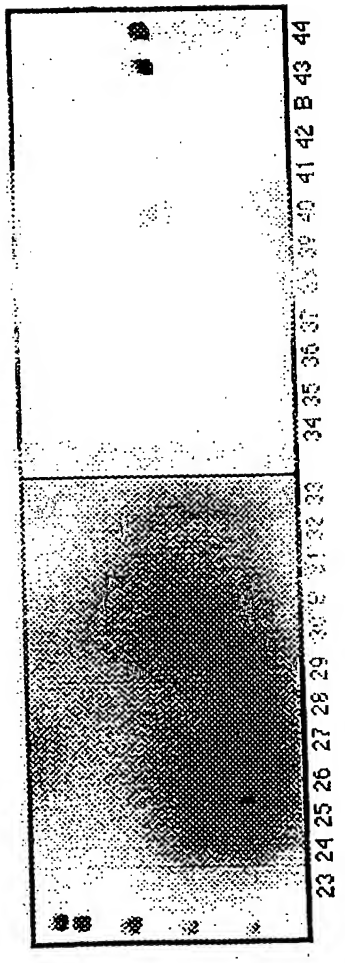
23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 B 43 44

FIG. 8B

# INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 97/19896

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ³ | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 90 08841 A (GENE TRAK SYSTEMS) 9 August 1990<br>see the whole document | 1-19 |
| A | WO 96 24686 A (BIO MERIEUX ;MABILAT CLAUDE (FR); SALLEN BRUNEHILD (FR)) 15 August 1996<br>see the whole document | 1-19 |
| A | CH 682 156 A (URS CANDRIAN ;BEDA FURRER (CH); CHRISTIANE HOEFELEIN (CH); JUERG L) 30 July 1993<br>see the whole document | 1-19 |
| A | WO 89 06699 A (PASTEUR INSTITUT) 27 July 1989<br>see the whole document | 1-19 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

³ Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 March 1998 | 24/03/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Müller, F |

Form PCT/ISA/210 (second sheet) (July 1992)

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9008841 A | 09-08-90 | AU 5188190 A | 24-08-90 |
| | | CA 2025236 A | 07-08-90 |
| | | EP 0418346 A | 27-03-91 |
| | | JP 3504677 T | 17-10-91 |
| | | US 5376528 A | 27-12-94 |
| WO 9624686 A | 15-08-96 | FR 2730234 A | 09-08-96 |
| | | CA 2210769 A | 15-08-96 |
| | | EP 0809710 A | 03-12-97 |
| CH 682156 A | 30-07-93 | NONE | |
| WO 8906699 A | 27-07-89 | AT 121460 T | 15-05-95 |
| | | DE 68922252 D | 24-05-95 |
| | | DE 68922252 T | 24-08-95 |
| | | EP 0355147 A | 28-02-90 |
| | | JP 2502880 T | 13-09-90 |
| | | US 5389513 A | 14-02-95 |
| | | US 5523205 A | 04-06-96 |

VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C12N 15/11

A1

(11) International Publication Number: WO 98/20160

(43) International Publication Date: 14 May 1998 (14.05.98)

(21) International Application Number: PCT/US97/19896

(22) International Filing Date: 3 November 1997 (03.11.97)

(30) Priority Data:

| | | |
|---|---|---|
| 08/745,228 | 8 November 1996 (08.11.96) | US |
| 08/766,439 | 12 December 1996 (12.12.96) | US |

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

| | |
|---|---|
| US | 08/745,288 (CIP) |
| Filed on | 8 November 1996 (08.11.96) |
| US | 08/766,439 (CIP) |
| Filed on | 12 December 1996 (12.12.96) |

(71) Applicant *(for all designated States except US)*: E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HAZEL, James, William [US/US]; 1181 Liberty Grove Road, Conowingo, MD 21918 (US). JENSEN, Mark, Anton [US/US]; 1176 Fielding Drive, West Chester, PA 19382 (US).

(74) Agent: MAJARIAN, William, R.; E.I. Du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**

*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: GENETIC MARKERS AND METHODS FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA SPP*

(57) Abstract

A method, diagnostic sequences and primers are provided that are useful in identifying the *Listeria monocytogenes* and *Listeria spp*. The method involves identifying an RAPD–amplified DNA fragment common to *Listeria monocytogenes*, then identifying the most conserved regions of that DNA fragments, and the preparing specific primers useful for detecting the presence of a marker within the fragment whereby that set of primers is then useful in the identification of all *Listeria monocytogenes*. Markers within the same fragment that are specific to the *Listeria* genus are also identified and are useful for the identification of all *Listeria spp*.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AL** | Albania | **ES** | Spain | **LS** | Lesotho | **SI** | Slovenia |
| **AM** | Armenia | **FI** | Finland | **LT** | Lithuania | **SK** | Slovakia |
| **AT** | Austria | **FR** | France | **LU** | Luxembourg | **SN** | Senegal |
| **AU** | Australia | **GA** | Gabon | **LV** | Latvia | **SZ** | Swaziland |
| **AZ** | Azerbaijan | **GB** | United Kingdom | **MC** | Monaco | **TD** | Chad |
| **BA** | Bosnia and Herzegovina | **GE** | Georgia | **MD** | Republic of Moldova | **TG** | Togo |
| **BB** | Barbados | **GH** | Ghana | **MG** | Madagascar | **TJ** | Tajikistan |
| **BE** | Belgium | **GN** | Guinea | **MK** | The former Yugoslav Republic of Macedonia | **TM** | Turkmenistan |
| **BF** | Burkina Faso | **GR** | Greece | | | **TR** | Turkey |
| **BG** | Bulgaria | **HU** | Hungary | **ML** | Mali | **TT** | Trinidad and Tobago |
| **BJ** | Benin | **IE** | Ireland | **MN** | Mongolia | **UA** | Ukraine |
| **BR** | Brazil | **IL** | Israel | **MR** | Mauritania | **UG** | Uganda |
| **BY** | Belarus | **IS** | Iceland | **MW** | Malawi | **US** | United States of America |
| **CA** | Canada | **IT** | Italy | **MX** | Mexico | **UZ** | Uzbekistan |
| **CF** | Central African Republic | **JP** | Japan | **NE** | Niger | **VN** | Viet Nam |
| **CG** | Congo | **KE** | Kenya | **NL** | Netherlands | **YU** | Yugoslavia |
| **CH** | Switzerland | **KG** | Kyrgyzstan | **NO** | Norway | **ZW** | Zimbabwe |
| **CI** | Côte d'Ivoire | **KP** | Democratic People's Republic of Korea | **NZ** | New Zealand | | |
| **CM** | Cameroon | | | **PL** | Poland | | |
| **CN** | China | **KR** | Republic of Korea | **PT** | Portugal | | |
| **CU** | Cuba | **KZ** | Kazakstan | **RO** | Romania | | |
| **CZ** | Czech Republic | **LC** | Saint Lucia | **RU** | Russian Federation | | |
| **DE** | Germany | **LI** | Liechtenstein | **SD** | Sudan | | |
| **DK** | Denmark | **LK** | Sri Lanka | **SE** | Sweden | | |
| **EE** | Estonia | **LR** | Liberia | **SG** | Singapore | | |